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(54) Backbone cyclic peptides, processes for their preparation and pharmaceutical compositions containing them

Peptide mit zyklischen Hauptketten, deren Herstellung und diese enthaltende pharmazeutische Zusammensetzungen

Peptides ayant une chaîne principale cyclique, leur préparation et compositions pharmaceutiques les contenant

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- **CHEMICAL ABSTRACTS, vol. 114, no. 11, 18 March 1991, Columbus, Ohio, US; abstract no. 102801b, BYK.G. ET AL. 'A new route to prepare conformationally restricted cyclic peptides as demonstrated by a potent NK-1 selective substance P analog' page 826 ; & PEPT.: CHEM.,STRUCT.BIOL.,PROC.AM.PEPT.SYMP.,11TH.,, 1990 pages 984 - 985 BYK G. ET AL.**
- **CHEMICAL ABSTRACTS, vol. 115, no. 19, 11 November 1991, Columbus, Ohio, US; abstract no. 208555k, GILON, CHAIM ET AL. 'Backbone-to-end cyclic tachykinins: a new approach to conformationally restricted peptides' page 1083 ;column LH ; & PEPT.1990, PROC.EUR.PEPT.SYMP.,21ST, 1991 pages 404 - 406**
- **CHEMICAL ABSTRACTS, vol. 117, no. 21, 23 November 1992, Columbus, Ohio, US; abstract no. 205809b, GILON C. ET AL. 'SAR studies of cycloseptide: Effects of cyclization and charge at position 6' page 170 ;column LH ; & PEPT.: CHEM.BIOL.,PROC.AM.PEPT.SYMP.,12TH, 1992 pages 476 - 477**
- **BIOPOLYMERS, vol.31, no.6, May 1991 pages 745 - 750 GILON C. ET AL. 'Backbone Cyclization: A New Method for Conferring Conformational Constraint on Peptides'**
- **Beilstein Registration No.1709709, abstract**
  Beilstein Registration No.5854650, abstract

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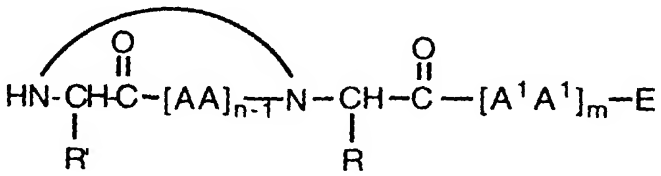
EP 0 564 739 B1

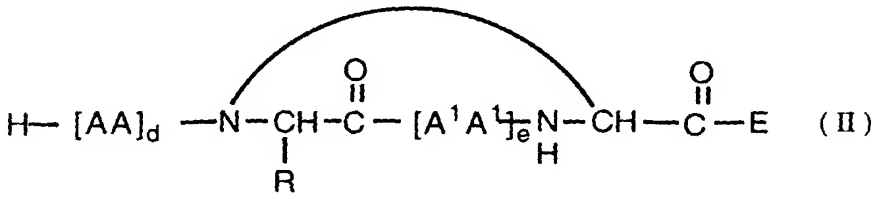
Description

[0001] The present invention relates to biologically active, backbone-cyclized peptides, to processes for their preparation and to pharmaceutical compositions containing them.

[0002] More particularly, the peptides of the invention are of general formulae (I) and (II):



(I)



(II)

wherein the substituents and circled line are as hereafter defined.

[0003] Following the pioneering work of R. Schwyzer [Ludecher, U., *et al*., Helv. Chim. Acta 54. 1637 (1971)] on gramicidin S, conformational restriction of peptides by medium and long range cyclization has been extensively employed. In addition to other modes of conformational restriction, such as configurational and structural alteration of amino acids, local backbone modifications, short-range cyclization etc., medium and long range cyclization [Hruby, V. J., Life Sci. 31, 189 (1982); Kessler, H., Angew. Chem. Int. Ed. Eng. ,21, 512 (1982); Schiller, P. W., in the "Peptides", Udenfriend, S., and Mejenhofer, J. Eds., Volume 6 p. 254 (1984); Veber, D. F. and Freidinger, R. M., Trends in Neurosci. 8, 392 (1985); Milner-White, E. J., Trends in Pharm. Sci. 10, 70 (1989)] is used for the following purposes: biologically active peptides are cyclized to achieve metabolic stability, to increase potency, to confer or improve receptor selectivity and to control bioavailability. The possibility of controlling these important pharmacological characteristics through cyclization of linear peptides prompted the use of medium and long range cyclization to convert natural bioactive peptides into peptidomimetic drugs. Cyclization also brings about structural constraints that enhance conformational homogeneity and facilitate conformational analysis [Kessler, H., Angew. Chem. Int. Ed. Eng., 21, 512 (1982)]. Moreover, the combination of structural rigidification-activity relationship studies and conformational analysis gives insight into the biologically active conformation of linear peptides.

[0004] In addition to other restrictive methods such as template associated synthetic proteins [Mutter, M., and Vuilleumier, S., Angew. Chem. Int. Ed. Eng., 101, 551(1982)], medium and long range cyclizations are also used for controlled stabilization of secondary and tertiary structures of proteins.

[0005] Small, linear, biologically active peptides usually exist in solution in a fast equilibrium of interchanging conformations [Kessler, H., Angew. Chem. Int. Ed. Eng., 21, 512 (1982)], which may lead to lack of receptor selectivity and metabolic susceptibility [Veber, D. F. and Freidinger, R. M., Trends in Neurosci. 8, 392 (1985)]. Moreover, this fast equilibrium also hampers attempts to determine their conformations in solution including the biologically active conformation. For example, a given linear peptide can exist in solution in a fast equilibrium of conformer A, which activates receptor A, conformer B, which activates receptor B and in an extended conformation C which fits into the active-site of a degrading enzyme. Structural modifications that slow down the fast equilibrium and reduce the conformational space impose conformational constraints on linear peptides [Kessler, H., Angew. Chem. Int. Ed. Eng., 21, 512 (1982)].

[0006] Ideally the best modification, from a biological standpoint, slows down the equilibrium or reduces the conformational space to such an extent that the peptide will interact only with receptor A and will not attain either the conformation that activates receptor B or the extended degradable conformation C. If an attempt is made to determine the active conformation, further restrictions might be needed. Experimental evidence for the validity of the theorem presented above can be found in the cases of the enkephalins, somatostatin, gonadotropin releasing hormone (GnRH), cholecystokinin (CCK), melanocyte stimulating hormone (αMSH)and many other peptides in which cyclization results in receptor selectivity and metabolic stability [Hruby, V. J, Life Sci. 31, 189 (1982); Kessler, H., Angew. Chem. Int. Ed. Eng., 21, 512 (1982); Schiller, P. W., in the "Peptides", Udenfriend, S., and Mejenhofer, J. Eds., Volume 6 p. 254 (1984);

Veber, D. F. and Freidinger, R. M., Trends in Neurosci. 8, 392 (1985); Milner-White, E. J., Trends in Pharm. Sci. 10, 70 (1989); Al-Obbeidi, F., *et al.*, J. Med. Chem., 32, 2555 (1989); Charpentier, B., *et al.*, J. Med. Chem., 32 1184 (1989); Rivier, J. E., *et al.*, in "peptides", Rivier, J. E., and Marshall, G. R. Eds., p. 33 (1990)] and in very few cases even allowed conformational analysis of the bioactive conformation [Kessler, H., Angew. Chem., 25 997 (1986)].

[0007] Conformationally restricted peptides containing medium and long range cyclizations have been mainly prepared following the same modes of cyclization of homodetic and heterodetic natural peptides. These include : a side-chain to side-chain cyclization (usually the formation of a lactam ring and/or an -S-S- bond through cyclization of functional groups already present in the native sequence or by substitution of other amino acids with Glu and Lys or Cys respectively); b end to end cyclization (previously called backbone to backbone cyclization [Manesis, N. J. and Goodman, M., Org. Chem., 52, 5331 (1987)]) and c side-chain to end groups cyclization.

[0008] The last mode of cyclization includes side-chain to amino end and side-chain to carboxyl end . The exact location, type and size of the ring (which can also be controlled by "spacers" [Manesis, N. J. and Goodman, M., Org. Chem., 52, 5331 (1987)]) to achieve maximum selectivity and activity is determined mainly by Structure-Activity-Relationship (SAR) considerations in conjunction with conformational analysis.

[0009] Despite the impressive success achieved hitherto with cyclic peptides, cyclization according to the above modes, caused in some the loss of biological activity especially if carried out in the "active region" of bioactive peptides. A typical example of the cyclization of peptides is the case of substance P (SP) and its related peptides the mammalian tachykinins neurokinin A (NKA) and neurokinin B (NKB). The tachykinins are short linear peptides (ten to eleven amino acid residues) which share the common carboxyl terminal sequence -Phe-X-Gly-Leu-Met-NH_2 (X =Phe or Val). The amino acid sequence of Substance P is the following:

	1	2	3	4	5	6	7	8	9	10	11
SP	Arg	Pro	Lys	Pro	Gln	Gln	Phe	Phe	Gly	Leu	Met-NH_2

(the positions of the amino acid residues in SP will be used in the description and Examples hereafter).

[0010] SP and the other tachykinins have been implicated in a variety of physiological functions, including transmission of pain stimuli, glandular secretion, intestinal motility, vasodilation, inflammatory pain reaction and a large variety of behavioral effects. The tachykinins activate three receptors NK-1, NK-2 and NK-3 [Trends Pharm. Sci. Receptor Nomenclature Supplement p. 25 (1990)]. The mammalian tachykinins are not selective in the sense that each one of them activates more then one receptor. Thus, for example NKB activates the NK-1 and the NK-3 receptors with comparable potency (EC_{50}(nM) of 4.2 and 1.3 respectively [Papir-Kricheli, D., *et al.*, Pain, 31, 263 (1987)]).

[0011] In addition to their lack of selectivity the mammalian tachykinins are degraded rapidly especially in in vivo assays [Wormser. U., *et al.*, in "peptides 1984" Ragnarsson, U., Ed. p. 359 (1985)].

[0012] The present inventors have prepared two conformationally constrained linear hexapeptide analogs of the carboxyl terminal sequence of SP, which incorporate N-alkylated amino acids, which showed receptor selectivity. The NK-3 selective analog Senktide [Laufer, R., *et al.*, J. Biol. Chem., 22, 10257 (1986)] (succ[Asp^6, N-Me Phe^8]SP_{6-11}, EC_50(nM) NK-3: 0.5, NK-2: >200,000, NK-1: 35,000) and the NK-1 selective analog WS Septide [Papir-Kricheli, D., *et al.*, Pain, 31, 263 (1987)] (Ac[Arg^6, Pro^9]SP_{6-11}, EC_{50}(nM) NK-1: 3.0, NK-2: >200,000, NK-3: >100,000). Senktide is metabolically stable in all tissues tested whereas WS Septide is metabolically unstable (half life of few minutes in liver, kidneys and parotid slices).

[0013] The present inventors have attempted to use the two selective analogs Senktide and WS Septide to elucidate the conformational requirements of the NK-3 and NK-1 receptors respectively. For this purpose they built tentative molecular models of Senktide and WS Septide based on NMR studies [Levian-Teitelbaum, D., *et al.*, Biopolymers, 28, 51 (1989)]. To correlate the molecular models of Senktide and WS Septide to the bioactive conformation, additional conformational constraints, such as cyclization could be imposed. Provided these more rigid analogs maintain their bioactivity and selectivity, they could be subjected to further conformational analysis. Attempts to cyclize the active region of SP, namely the C-terminal hexapeptide, resulted in biologically inactive compounds [Neubert, K., *et al.*, Pharmazie 40, 617 (1985); Chassing, G., *et al.*, in "peptides 1984" Ragnarsson, U., Ed. p. 345 (1985); Sandberg, B. E. B., *et al.*, in "peptides 1984" Ragnarsson, U., Ed. p. 369 (1985); Theodoropoulos, D., *et al.*, J. Med. Chem., 28, 1536 (1985); Drmen, P. S., *et al.*, Biochem. Biophys. Res. Commun., 127, 656 (1985); Mutulis, F., *et al.*, Bioorg. Chim., 11, 1276 (1985)].

[0014] In a theoretical article [Gilon, C., *et al.*, Biopolymers, 31, 745 (1991), see also Chem. Abstracts 114, No. 102801, Vol. 115, No. 208555, Vol. 117, No. 205809] the inventors presented a new general concept of cyclization, employing WS Septide as model peptide. The proposed new cyclization method has been termed "backbone cyclization" and is discussed in detail in said article. Few of the cyclized peptides subject of the present invention are generally disclosed in said article, however the article does not propose any method for the preparation of the present com-

pounds.

[0015] Subsequent work by the present group of inventors led to the preparation of the present novel cyclized peptides. Notwithstanding the fact that the objects to be achieved by cyclization were well defined, and although the theoretical SAR consideration were taken into account, great efforts were involved in aiming at the novel, biologically active and selective peptides. The SP analogs which will be specifically exemplified hereafter are to be considered as models for the universal cyclization methods disclosed, and the cyclic peptides prepared thereby.

[0016] Neuropeptides control a wide range of endocrinological, motoric and behavioral activities [Krieger, D. T., Science 222, 975-978 (1983); Snyder, S. H. Science 209, 976 (1980)]. These substances mediate a large variety of physiological functions including analgesia, appetite control, thirst, body temperature, mood, learning, neurological behavior, pain and modulation of immune response. Thus derivatives of the naturally occurring neuropeptides, both as agonists or antagonists, have a great potential in the prevention and treatment of many neurological and behavioral disorders.

[0017] However, despite many attempts, there are only few ligands which are used therapeutically. The prior attempts were mainly directed at imitating the biological activity of the naturally occurring peptides. In order to design and synthesize a therapeutic agent which would control and/or modulate said activity, many factors are to be taken into account. Information regarding the bioactive conformation of the peptide is important. The peptides can occur in different conformations, each of which is specific for a different receptor. In addition to the desired biological activity, lack of conformational stability would result in association with undesired receptors, and with undesired side-effects. The bioactive conformation of only vary few of the many known neuropeptides has yet been established.

[0018] Neuropeptides undergo fast proteolytic degradation in the gastrointestinal tract, in blood and in other tissues. The pharmacokinetics of neuropeptides in the synapse are completely different from those of a therapeutic peptide. In the synapse, the time required for the neuropeptides to accomplish their function is very short due to the short distance involved. The endogenous proteases which are responsible for terminating the activity of a certain neuropeptide are the only ones present in the vicinity, and they degrade the peptide immediately after completion of its function. In order to devise a therapeutic peptide , its structure should be stabilized against the proteases responsible for its degradation, as well as against other proteases present in the GI tract, blood and other tissues.

[0019] Natural peptides do not easily cross the GI/blood and blood/brain barriers. This problem cannot be secluded from that of metabolic stability. Crossing both the GI/blood and blood/brain barriers are key steps when considering using a peptide as a drug. While several peptides were successfully stabilized against degradation, their bioavailability, namely crossing the blood/brain barrier remained a mystery. In addition, different receptors induce different physiological activities, therefore triggering an undesired receptor by the peptide would cause undesired side-effects. The common hypothesis is that a linear peptide is in a state of fast equilibrium between a large number of conformations, some of which would activate undesired receptors. Also, the peptide has to be in the right conformation in order to undergo enzymatic degradation. Only the desired conformation, termed the bioactive conformation, would activate the desired receptor and result in only the specific biological activity which is wanted.

[0020] These problems of naturally occurring peptides, such as, for example, neuropeptides are of great significance and constitute major problems in applying the peptide as a drug , as well as understanding the pharmacological and molecular interaction between the peptide and its receptor.

[0021] Successful prior art results, for example the analgesic peptidomimetic opiates, which are "imitations" of endogenous enkephalins, were achieved through works which were more random than rationally planned and did not take into consideration all the factors described above.

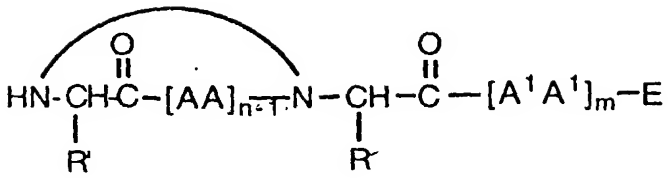
BRIEF DESCRIPTION OF FIGURES

[0022]

Figure 1 Illustrates the concept of backbone cyclization;
Figure 2 shows degradation of WS- Septide (prior art) and peptide Ia by parotid slices; and
Figure 3 shows the dose response curves of WS-Septide (prior art) and peptide Ia

SUMMARY AND DESCRIPTION OF THE INVENTION

[0023] According to one aspect of the invention there is provided a process for the preparation of backbone cyclized, biologically active polypeptides of the general formula:

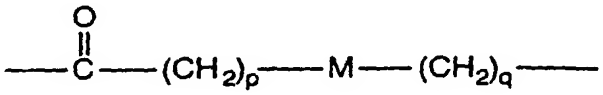


(I)

wherein

n designates an integer of from 1 to 10;
m designates an integer of from 1 to 10;

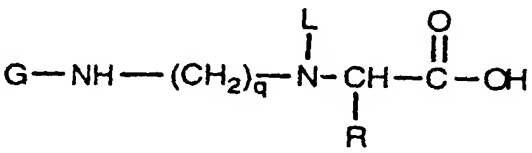
[AA] designates a naturally occurring or synthetic amino acid residue, wherein the amino acid residues in case n is greater than 1 may be the same or different;
[A^1 A^1] designates a naturally occurring or synthetic amino acid residue, wherein the amino acid residues in case m is greater than 1 may be the same or different;
R and R' designate a naturally occurring or synthetic amino acid side-chain;
E represents a hydroxyl, or a carboxyl protecting group which is standard in peptide synthesis [e.g. Schroeder *et al.*, "The Peptides", Vol. I, Academic Press, 1965; or Bodanszky, "Principles of Peptide Synthesis", Springer-Verlag, 1984; or Bodanszky *et al.*, "the Practice of Peptide Synthesis", Springer-Verlag, 1984; or McOmie (ed.), "Protective Groups in Organic Chemistry", Plenum Press, 1973; or Greene, "Protective Groups in Organic Synthesis", Wiley-Interscience, 1981; or Barany and Merrifield, in "The Peptides: Analysis, Synthesis and Biology", Vol. 2, Chapter 1, Academic Press, 1980], selected from alkoxy, substituted alkoxy or aryloxy, or a blocking group which may be the same as the carboxyl group or an amino or substituted amino group, wherein the carboxyl protecting or blocking group may be optionally covalently attached to an insoluble polymeric support, and the circled line designates a spacer group of the formula:



(III)

wherein M is selected from the group consisting of -S-S-, -CO-NH- and -S- and p and q, which may be the same or different, each designates an integer of from 2 to 10, which process comprises the steps of

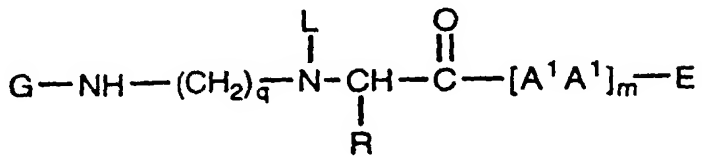
(a) reacting a compound of the formula:



(Va)

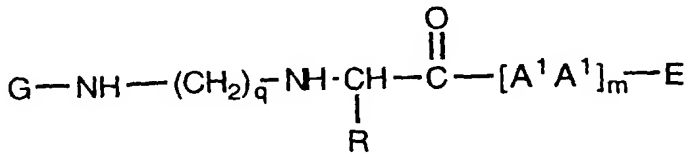
wherein q represents an integer of from 2 to 10, G and L, which may be the same or different, each represents a protecting group conventional in peptide synthesis [e.g. Schroeder *et al.*, "The Peptides, Vol. I, Academic Press, 1965; or Bodanszky, "Principles of Peptide Synthesis", Springer-Verlag, 1984; or Bodanszky *et al.*, "the Practice of Peptide Synthesis", Springer-Verlag, 1984; or McOmie (ed.), "Protective Groups in Organic Chemistry", Plenum Press, 1973; or Greene, "Protective Groups in Organic Synthesis," Wiley-Interscience, 1981; or Barany and Merrifield, in "The Peptides: Analysis, Synthesis and Biology", Vol. 2, Chapter 1, Academic Press, 1980], with an amino acid or a polypeptide of the formula H_2N-$[AA]_m$-CO-E wherein m is an integer from 1 to 10 and AA represents a naturally occurring or synthetic amino acid residue, the amino acid residues in case m is greater than 1 being the same or different, and E represents a carboxyl protecting group selected from

alkoxy, substituted alkoxy or aryloxy, or a blocking group which may be the same or an amine or substituted amine group, wherein the carboxyl protecting or blocking group may be optionally covalently attached to an insoluble polymeric support, to give a compound of the formula:



(VIa)

b) selectively removing from the compound of formula VIa, protecting group L, to give a compound of the formula:

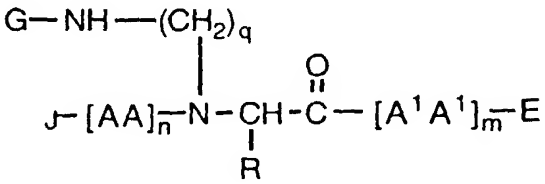


(VIIa)

c) reacting the compound of formula VIIa with an amino acid or peptide of the formula:

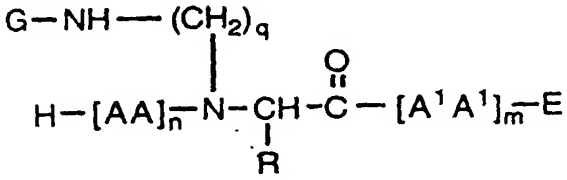
(VIIb)

wherein n is an integer of from 1 to 10, [AA] represents a naturally occurring or synthetic amino acid residue, the amino acid residues in case n is greater than 1 being the same or different and J represents a protecting group conventional in peptide synthesis to give a compound of the formula:



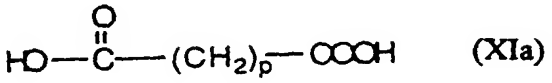
(IXa)

d) selectively removing the protecting group J to give a compound of the formula:



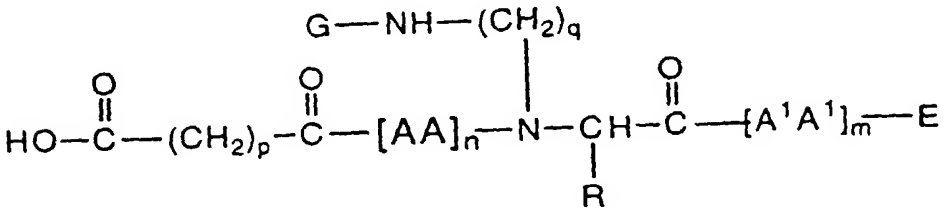
(Xa)

e) reacting the compound of formula Xa with a compound of the formula:



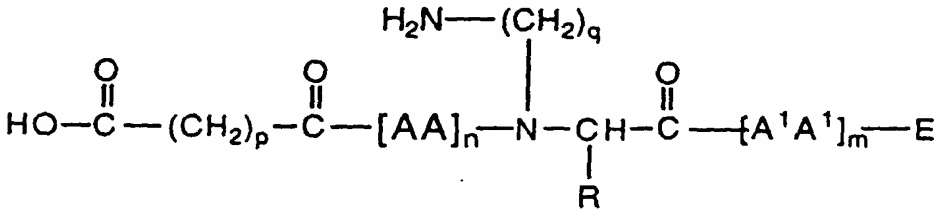
(XIa)

wherein p is as defined above to give a compound of the formula:



(XIIa)

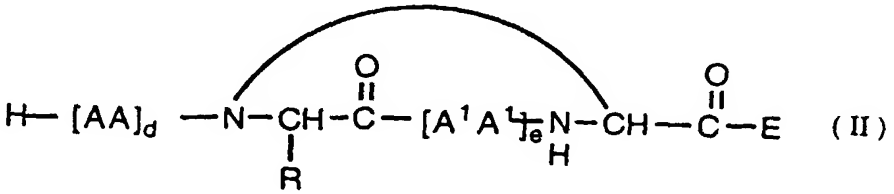
f) selectively removing from the compound XIIa the protecting group G to give a compound of the formula:



(XIIIa)

g) reacting the compound of formula XIIIa with a suitable coupling agent selected from the group consisting of dicyclohexylcarbodiimide (DCC), bis(2-oxo-3-oxazolidinyl) phosphinic chloride (BOP-Cl), benzotriazolyl-N-oxytrisdimethylaminophosphonium hexafluoro phosphate (BOP), 1-oxo-1-chlorophospholane (Cpt-Cl), and a mixture of DCC and hydroxybenzotriazole (HOBT) and removing other side-chain protecting groups to give a compound of the general formula I where m is an integer from 1 to 10.

[0024] The invention further comprises a process for producing backbone cyclized, biologically active polypeptides of the general formula:



(II)

wherein

d is 0 or an integer of from 1 to 10 and e is an integer of from 1 to 10;
[AA] designates a naturally occurring or synthetic amino acid residue, wherein the amino acid residues in case e is

greater than 1 may be the same or different;

[A^1 A^1] designates a naturally occurring or synthetic amino acid residue, wherein the amino acid residues in case d is greater than 1 may be the same or different;

R designates a naturally occurring or synthetic amino acid side-chain;

E represents a hydroxyl, or a carboxyl protecting group which is standard in peptide synthesis, preferably selected from alkoxy, substituted alkoxy or aryloxy, or a blocking group which may be the same as the carboxyl group or an amino or substituted amino group, wherein the carboxyl protecting or blocking group may be optionally covalently attached to an insoluble polymeric support, and the circled line designates a spacer group of the formula:

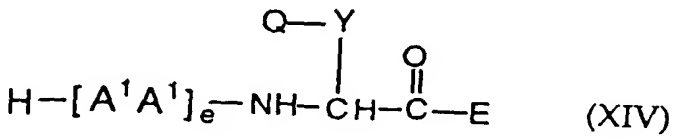
$$—(CH_2)_p—(M)_x—Y \qquad (IV)$$

wherein p is as defined above and M is an amino or a carboxyl group or a sulfur atom, x is 0 or 1, and Y designates the side-chain of a backbone amino acid.

[0025] In the compounds of formula I, M as defined in formula III is preferably an amide or -S-S- group.

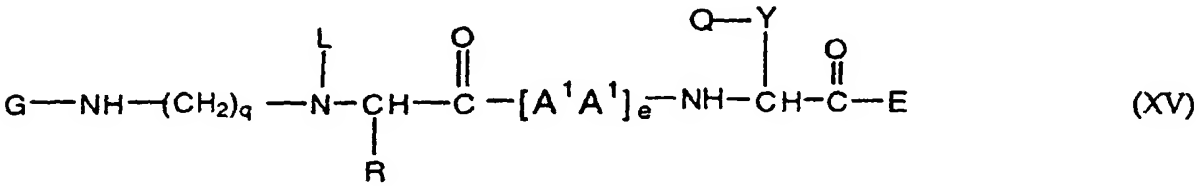
[0026] In the compounds of formula II, Y as defined in formula IV is preferably the side chain of homocysteine, x as defined in formula IV is preferably 0, and in case x is 1, M is preferably a sulfur atom, which process comprises:

a) reacting a compound of the formula:



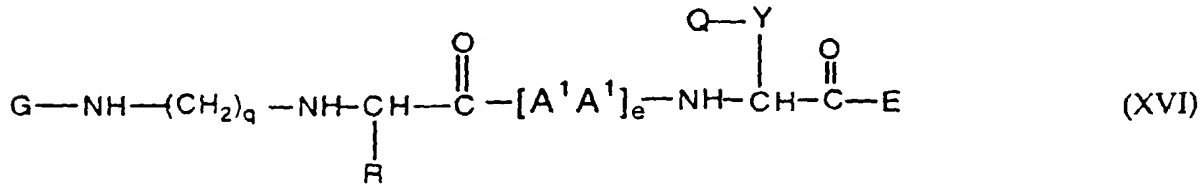
(XIV)

wherein E and e are as defined above, [A^1A^1] represent a naturally occurring or synthetic amino acid residue and Y represents a side-chain of naturally occurring or synthetic amino acid and Q represents a protecting group conventional for peptide synthesis with a compound of formula Va, to give a compound of the formula:



(XV)

b) selectively removing from the compound of formula XV protecting group L, to give a compound of the formula:

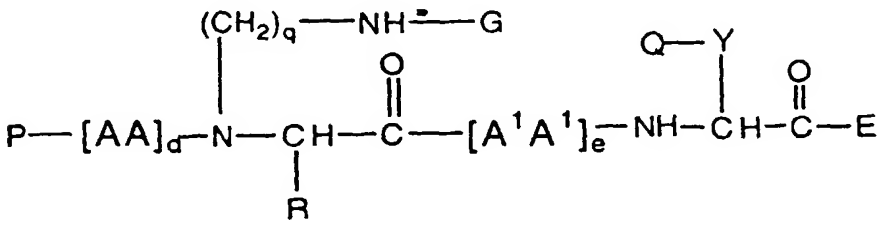


(XVI)

c) reacting the compound of formula XVI with an amino acid or peptide of the formula:

$$P—[AA]_d—CH \qquad (XVII)$$

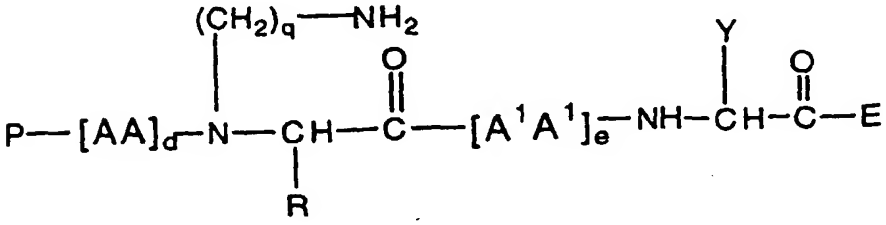
wherein d is an integer of from 1 to 10 and [AA] represents a naturally occurring or synthetic amino acid residue, the amino acid residues in case d is greater than 1 being the same or different, and P represents a protecting group conventional in peptide synthesis, to give a compound of the formula:



(XVIII)

d)

(i) in case Y is a carboxyl bearing side-chain, selectively removing the protecting groups G and Q from compound XVIII to give a compound of the formula:}

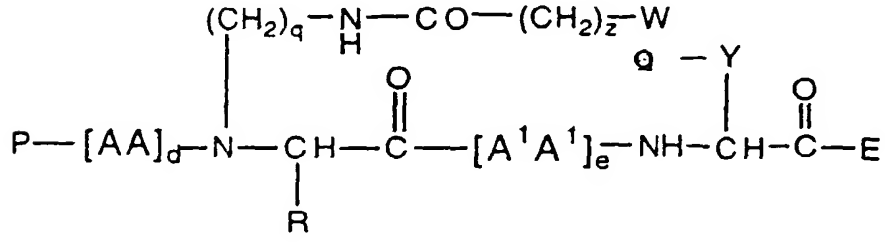


(XIX)

cyclizing the compound XIX by reacting it with the coupling agent according to step g) above and removing protecting group P and other side-chain protecting groups to give a compound of the general formula; or
(ii) in case Y is a side-chain bearing a nucleophilic functional group other than carboxyl, selectively removing protecting group G, reacting the resulting compound with a compound of the formula:

$$HOOC—(CH_2)_z—W \qquad (XX)$$

wherein z is an integer of from 1 to 10 and W is a function bearing group capable of reacting with said nucleophilic group, to give a compound of the formula:



(XXI)

selectively removing the protecting group Q, whereupon cyclization occurs, and removing the protecting group P and other side-chain protecting groups from compound XXI to give a compound of the general formula II.

[0027] In the compounds of both general formulae I and II, E is preferably an amino group.

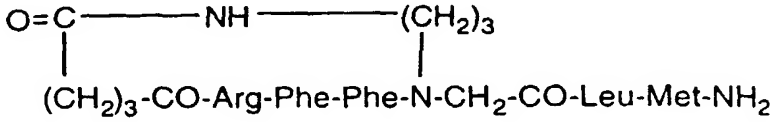
[0028] Throughout this application the various groups

$$—[AA]_y—$$

designate an amino-acid residue in case y is 1, or a peptide chain in case y is greater than 1. It is to be understood, that in case y is defined as zero, the whole group is absent.

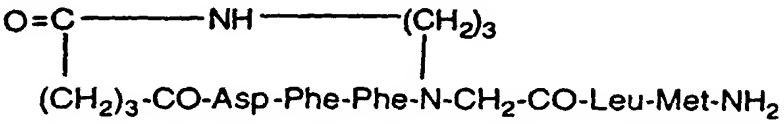
[0029] Preferred specific cyclized peptides, which serve herein as a model for the present cyclopeptides and the processes for their preparation are homologs of neuropeptides, more specifically of Substance P. Preferred peptides according to formula I are:

Ib



R= $(CH_2)_3$ NH-C(=NH)NH_2 ; n= 3; $[AA]_{n-1}$ = Phe-Phe; m= 2; $[A^1A^1]_m$ = Leu-Met-NH_2; circled line = CO-$(CH_2)_3$ -CO-NH-$(CH_2)_3$.

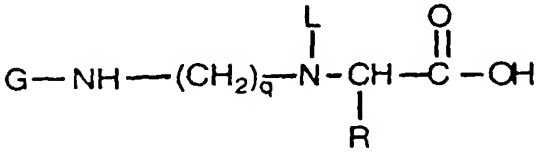
Ic



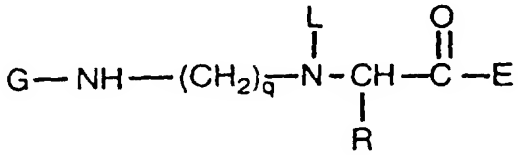
R'= CH_2 -COOH ; n= 3; $[AA]_{n-1}$ = Phe-Phe; m= 2; $[A^1A^1]$m = Leu-Met-NH_2; circled line = CO-$(CH_2)_3$ CO-NH-$(CH_2)_3$.

[0030] Backbone cyclization according to the present invention involves the joining of the N (and/or the C) atoms in the peptidic backbone. The concept of N-backbone cyclization is shown in Figure 1. Thus, for example, to achieve N-backbone cyclization, hydrogens of the peptide bond are replaced by (-functionalized alkylene chains that can then be either connected to side-chains or ends or interconnected to form the desired cyclic peptide.

[0031] Compounds of the general formulae Va and Vb may be produced:



(Va)



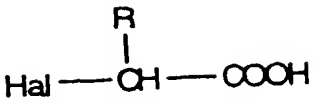
(Vb)

wherein the substituents are as defined above. These compounds are intermediates in the processes for the preparation of the cyclized peptides of the invention.

[0032] Within ambit of the invention are also processes for the preparation of the compounds Va and Vb.

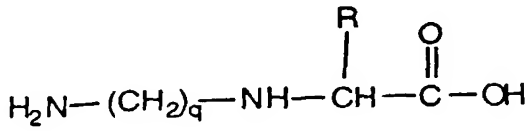
[0033] According to one embodiment, compounds of general formula Va are prepared as follows:

(a) an α-halocarboxylic acid of the formula:



(XXII)

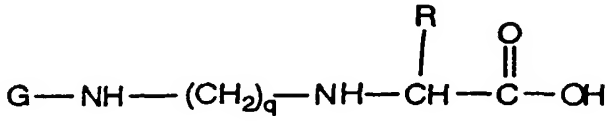
wherein R is as defined above and Hal is chlorine, bromine or iodine, is reacted with an alkylene diamine of the formula: $H_2N—(CH_2)_q—NH_2$ (XXIII) wherein q is as defined above, to give a compound of the formula:



(XXIV)

(b)

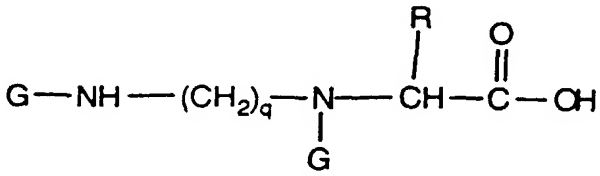
(i) reacting the compound of formula XXIV with suitable reagent containing the group G by standard methods of peptide synthesis, where G is as defined above, to give a compound of the formula:



(XXVI)

and reacting the compound of formula (XXVI) with a reagent containing L by standard methods of peptide synthesis, where L as defined above, to give a compound of the formula Va; or

(ii) reacting the compound of formula XXIV with a suitable reagent containing G by standard methods of peptide synthesis, where G as defined above to give a compound of the formula:



(XXVIII)

selectively removing the protecting group G from the secondary amino function to give a compound of the general formula XXVI.

[0034] Compounds of general formula Vb as defined herein wherein q represents an integer of from 2 to 10, G and L, which may be the same or different, each represents hydrogen and/or a protecting group conventional in peptide synthesis, and E represents a hydroxyl, or a carboxyl protecting group which is standard in peptide synthesis, preferably selected from alkoxy, substituted alkoxy or aryloxy, or a blocking group which may be the same as the carboxyl group or an amino or substituted amino group, wherein the carboxyl protecting or blocking group may be optionally covalently attached to an insoluble polymeric support may be prepared by;

(i) reacting the free acid Va defined herein with the appropriate reagent containing the group E by standard methods of peptide synthesis, wherein E as defined above, to give the ester, amide, or covalent bond with the polymeric support; or

(ii) from compounds of the formula VIb defined herein by reacting this free acid with the appropriate reagent containing the group E by standard methods of peptide synthesis, wherein E as defined above, to give the ester, amide or covalent bond with the polymeric support; the compound of formula VIb is obtained from compound XXVI by reacting this free acid with the appropriate reagent containing the group E as defined above, to give the ester, amide, or covalent bond with the polymeric support.

[0035] In the compounds of formulae V to XXVIII described above, the protecting groups G, L or J preferably Boc, Fmoc or Z, and the protecting group Q is preferably, in addition to the above, Bzl, ACM or t-Butyl.

[0036] In the processes described above, the coupling agents are preferably dicyclohexylcarbodiimide (DCC), bis(2-oxo-3-oxazolidinyl) phosphinic chloride (BOP-Cl, benzotriazolyl-N-oxytrisdimethylaminophosphonium hexafluoro phosphate (BOP), 1-oxo-1-chlorophospholane (Cpt-Cl), and a mixture of DCC and hydroxybenzotriazole (HOBT).

[0037] The invention also relates to pharmaceutical compositions comprising as active ingredients the cyclized peptides of the invention which may be used where selective bioaction of the peptides is required. The cyclic peptides of the invention can be used as stable and selective agonists of natural peptides, for example neuropeptides such as human tachykinins. The pharmaceutical compositions of the invention may be used for treatment of various disease states where involvement of tachykinins is implied. As such, they may be used, for example, for treatment of various inflammatory diseases, psoriasis, rheumatoid arthritis, and neurological disorders such as Familial Dysautonomia, Parkinsonism, Alzheimer's disease, and Retardive Dyskinesia. The present preparations may also be useful as analgesics. The preparations may also be used as hypotensive agents and respiratory stimulants.

[0038] The novel compositions contain in addition to the active ingredient conventional pharmaceutically acceptable carriers, diluents and the like. Solid compositions for oral administration such as tablets, pills, capsules or the like may be prepared by mixing the active ingredient with conventional, pharmaceutically acceptable ingredients such as corn starch, lactose, sucrose, sorbitol, talc, stearic acid, magnesium stearate, dicalcium phosphate , and gums, with pharmaceutically acceptable diluents. The tablets or pills can be coated or otherwise compounded with pharmaceutically acceptable materials known in the art to provide dosage form affording prolonged action or sustained release. Other solid compositions can be prepared as suppositories, for rectal administration. Liquid forms may be prepared for oral administration or for injection, the term including sub-cutaneous, transdermal, intravenous , intrathecal, etc. administration. The liquid compositions include aqueous solutions, flavoured syrups, aqueous or oily suspensions, flavoured emulsions with edible oils, as well as elixirs and similar pharmaceutical vehicles. In addition, the compositions of the present invention may be prepared as aerosols, for intra-nasal and like administration.

[0039] The active dose for humans is generally in the range of from 0.05 mg to about 50 mg per kg body weight, in a regimen of 1-4 times a day. However, administration every two days or more is also possible. Specific dosages would be determined by the attending physician according to the disease to be treated, method of administration, patient's age, weight, counterindications and the like.

EXAMPLES

SYNTHESIS

GENERAL - MATERIALS AND METHODS

[0040] Alkylene diamines were purchased from Merck Schuchatdt and were used without further purification. a-Chloro carboxylic acids were synthesized from the corresponding amino acids [Kopepenhoefer, B; Schurig, V,. Organic Synthesis, 66, 151 (1987), Heathcock, C. H. Ed; Organic Synthesis Inc. USA]. a- Bromo carboxylic acids were prepared according to modified procedure [Kopepenhoefer, B; Schurig, V,. Organic Synthesis, 66, 151 (1987), Heathcock, C. H. Ed; Organic Synthesis Inc. USA] (5N HBr was used instead of 5N HCl). Benzyl p-nitrophenyl carbonate and BOP-Cl were purchased from Aldrich. BOP-Cl was purified according to the known procedure [Van Der Awers, C. and Anteumis, M. J. O. Int. J. Peptide Protein Res. 29, 574 (1987)]. BOP reagent was purchased from Richelieu Canada. Thionyl chloride was refluxed and distilled over flax oil. All solvents were analytically pure and used without further purification. HPLC was performed on a Merck Hitachi 655A equipped with a LC-5000 gradient pump and UV-VIS detector with tunable wave length set at 220 nm. The flow was fixed at 1 mL/min and the eluants were water (+0.05% TFA), MeOH and MeCN. The columns were Lichrosphere RP-18 or RP-8 15cm x 4.2 mm ID from Merck. Optical purity was checked on ChiraSpher^{R} column from Merck (5m, 25cmX4mm ID). The flow was fixed at 1 mL/min and the eluant was a mixture of n-hexane-dioxane-i-propanol 50/44/5. The detector was set to 254 nm. Melting points were measured on a Thomas Hoover capillary machine and optical activity was measured on a Perkin Elmer-141 polarimeter in a 10 cm length cell with a sodium lamp at 25° C . Microanalysis was carried out at the microanalytical department of The Hebrew University, Jerusalem. ^{1}H NMR spectra of building units and dipeptides were recorded on a Bruker WP-200 pulsed FT spectrometer. Samples were dissolved in $CDCl_3$. Chemical shifts are in ppm relative to TMS internal standard. ^{1}H NMR

spectra of peptide Ib were recorded on Bruker AMX-500 and 600 spectrometers, operating at 500 and 600 MHz proton resonance frequencies. Data were processed on Bruker X32 work station using the UXNMR program. 31 mg of peptide were dissolved in DMSO-d_6 from Aldrich in a 5 mm NMR tube. Spectra were recorded at 303 K. The assignment of the proton resonances was carried out following standard procedures [Wutrich, K., NMR of Proteins and Nucleic Acids, John Wiley, NY, 1986] using the homonuclear NOESY, ROESY, TOCSY AND E. COSY techniques. FAB-MS was determined on a ZAB-3HF FAB/tandem mass spectrometer or on an API/// LC/MS/MS.

SYNTHESIS OF BUILDING UNITS AND DIPEPTIDES

Method A Preparation of N-(ω-amino alkylene) amino acids.

[0041] The appropriate alkylene diamine (15.8 mol) was rapidly stirred at 4°C (if the alkylene diamine is solid it was dissolved in 500 mL CH_2Cl_2) while the a-halogeno carboxylic acid (1.6 mol) was added portionwise ensuring that each addition had solubilized. The reaction was then stirred at 25° C for 48 h and evaporated in vacuo (60° C). To the resulting paste, a solution of DMSO/Ether/Ethanol (3:1:1) 500 mL was added and the mixture left overnight in the freezer. The precipitated zwitterions were collected by filtration on sintered glass and washed with ethanol and ether. In some cases the products were obtained as the dihydrochloride salts rather than the zwitterions (structures and chemical data see Table I). The optical purity of compounds 6 and 7 was checked on their fully protected derivatives 14 and 15 (see method C and Table II).

Method B Selective protection of N-(ω-amino alkylene) amino acids.

[0042] A solution of benzyl p-nitrophenyl carbonate (0.605 mol) in dioxane (1.3 L) was added dropwise to a stirred solution of the N-(w-amino alkylene) amino acid (0.4 mol) in 50% aqueous dioxane (2.6 L). The mixture was maintained at pH=11 (with 2 N NaOH in an automatic titration). After stirring for 24 h at room temperature the mixture was evaporated to dryness, dissolved in H_2O (1.2 L) and filtered. The filtrate was extracted with EtOAc (2x1 L) and the aqueous layer was cooled in a water-ice bath and acidified to pH=5.5 with 6N HCl. After extraction with ether (2x1 L), the aqueous layer was acidified (pH=1 with concentrated HCl), evaporated to dryness and reevaporated from i-POH. In one case (starting material 1 Table I) crystallization from i-PrOH gave the acid 8 (Table II). When this procedure was applied to materials 2 - 7 the products were oils or were obtained in low yield. In these cases the crude mono protected N-(Z-ω-amino alkylene) amino acids were esterified according to method C below. This procedure increased considerably the overall yields of the diprotected N-(w-amino alkylene) amino acids. Alternatively, the crude mono protected N-(w-amino alkylene) amino acids could be protected on the N(with Boc (method H below), then the N(-Z cleaved (method K below) and the N(protected with Fmoc (method J) (structures and chemical data see Table II).

Method C Esterification of mono protected N-(w-amino alkylene) amino acids.

[0043] Crude N-(Z-ω-amino alkylene) amino acids (40 mmol) were suspended and stirred in anhydrous MeOH (600 mL) and dry HCl (H_2SO_4 trap) was bubbled for 1 h. The stirring was continued for 1 h at room temperature and the MeOH was evaporated in vacuo.

[0044] The crude product was dissolved in water (500 mL) and washed with EtOAc (2x500 mL). The pH of the water was raised to 8 (saturated $NaHCO_3$) and extracted with EtOAc (3x300 mL). The organic phase was dried over $MgSO_4$ and evaporated to dryness in vacuo. (structures and chemical data see Tables II and III). The optical purity of the two enantiomers 14 and 15 was checked on ChiraSpher^R column. Each compound gave only one peak at different k' whereas a mixture gave two peaks with the same k' corresponding to those of the pure compounds.

Method D Preparation of N-(w-Boc-amino alkylene) Gly.

1. Preparation of Boc-alkylene diamine.

[0045] The appropriate alkylene diamine (1 mol) was dissolved in $CHCl_3$ (1 L). The stirred solution was cooled in an ice bath and $(Boc)_2O$ (0.1 mol in 0.5 L $CHCl_3$) was added dropwise. The solution was stirred additional 24 h at room temperature and the solvent evaporated to dryness in vacuo. The resulting oil was dissolved in ether (0.5 L) and washed with brine (6X200 mL). The etheral layer was dried on $MgSO_4$ and evaporated to dryness in vacuo. The resulting oil was dried on P_2O_5 in vacuo.

[0046] 2. The appropriate mono Boc-alkylene diamine (1 mol) was rapidly stirred at 0°C while a- chloro acetic acid (0.1 mol) was added portionwise ensuring that each addition had solubilized. The mixture was left overnight at room temperature, then ether was added (50 mL) and the precipitate collected by filtration, washed with ether (3x50 mL) and

dried over P_2O_5. The solid dissolved in water (pH=10) and lyophilized. The solid was dissolved in water (80 mL) and the N(protected with Fmoc (method J) to give products 16 & 17 (see Tables II and III).

Method E Selective deprotection of Z-N-(Z-ω-amino alkylene) amino acids.

[0047] N-(w-amino alkylene) amino acids were reacted with 2 equivalents of Z-Cl according to method L . The di-Z products (30 mmol) were dissolved in neat $SOCl_2$ (50 mL) and waned to 60°C for 0.5 h. The solution was evaporated in vacuo and HCl 2N (100 mL) was added to the resulting paste. The mixture was stirred during 3 h and washed with ether (3x100 mL). The pH of the solution was adjusted to 9 and the N(amino group protected with Boc in situ (method H) . The N(Z-protecting group was removed by catalytic hydrogenation (method K) and reprotected with Fmoc (method J) (see Tables II and III). Alternately, MeOH was added to the paste instead of HCl to give, after evaporation in vacuo, esters 9 - 15 (scheme 2 and table II).

Method F Coupling with BOP-Cl.

[0048] BOP-Cl (1.1 mmol) was added to a stirred solution of the diprotected N-(w-amino alkylene) amino acids (1 mmol) followed by 1.2 mmol of DIEA in 10 mL MeCN at - 15°C. The solution was stirred for 20 mm at -15° C and the amino acid ester salt (1 mmol) was added in 210 mL of MeCN with 1.1 mmol of DIEA. The stirring was continued overnight at 0° C. The solvent was evaporated under vacua and the crude product dissolved in EtOAc and washed with saturated solutions of $KHSO_4$ (2x 300 mL), $NaHCO_3$ (2x300 mL) and brine (2x300 mL). The organic phase was dried over $MgSO_4$ and evaporated in vacuo to dryness. (see Table IV)

Method G Coupling with BOP.

[0049] To a stirred solution of the amino acid ester component (1 mmol) in CH_2Cl_2 (10 mL) were added BOP reagent (1.1mmol) and the diprotected N-(w-amino alkylene) amino acids (1.1mmol) and DIEA (3 mmol) at room temperature. After 15 min the pH was checked for basicity (in cases where the pH was lower than pH=9, more DIEA was added) and the reaction mixture left for 1 h at room temperature. The solvent was evaporated under vacuum and the crude product dissolved in EtOAc (100 mL) and washed as described in method F above (see Table IV).

Method H Preparation of Boc-amino acids [Nagasawa *et al* ., Bull. Chem. Soc. Jap., 46, 1269 (1973)].

[0050] Amino acid (0.1 mol) was dissolved in NaOH (1N, 200 mL) and dioxane (200 mL) added. The mixture was stirred in an ice bath and a solution of $(Boc)_2O$ (0.14 mol) in dioxane (200 mL) was added dropwise while the pH was maintained at 9. The mixture was left stirring at room temperature over night. The dioxane was evaporated in vacuo and the water solution washed with ether (3X150 mL), cooled and acidified with saturated $KHSO_4$ solution to pH 3. The precipitate was collected by filtration, washed with cold water and dried on P_2O_5 in vacuo to constant weight. If upon acidification an oil was formed, it was extracted with EtOAc (3X150 mL) which was washed with saturated NaCl, dried over $MgSO_4$ and evaporated to dryness. After drying over P_2O_5 , the residue was crystallizes from EtOAc/petrol ether.

Method J Preparation of Fmoc-amino acids [Sivanandaiah, K. M.; Rangaragn, M. S., Ind. J. Chem., 25(B), 1045 (1986)].

[0051] A solution of Fmoc-OSu (0.024 mol) in MeCN (25 mL) was added at once to a stirred aqueous solution of amino acid (0.025 mol) adjusted to pH 9 with TEA. The pH was maintained at 8.5-9 with TEA. After 15 min the pH stabilized and the reaction mixture was left another 15 min. The MeCN was evaporated in vacuo, the pH adjusted to 3 with saturated $KHSO_4$ and the precipitate collected by filtration, washed with cold water and dried over P_2O_5 to constant weight. If upon acidification an oil formed it was treated as in method H.

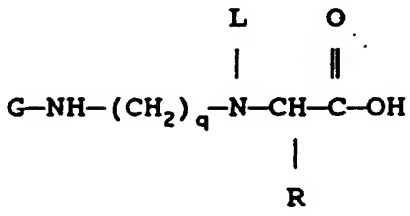
Method K Removal of the Z protecting group [Anwer, M. K.; Spatola, A. F., Synthesis, 929 (1980)].

[0052] To a solution of Z-amino acid (1 gr) dissolved in MeOH (5mL), Pd/C 10% (0.1 gr) and ammonium formate (1 gr) were added with stirring. The advance of the reaction was followed by HPLC. After completion (~2 h), the catalyst was removed by filtration and the filtrate evaporated to dryness in vacuo. The residue was dissolved in water which were lyophilized.

Method L Preparation of Z-amino acids [Bergman, M.; Zervas, L., Ber. 65, 1192 (1932)].

[0053] Z-amino acids were prepared according to method H, but Z-Cl was used instead of $(Boc)_2O$.

Table I. Data for compounds of structure Va [G=H;L=H]

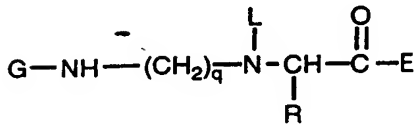


(Va)

Serial No.	n =	R	method	% yield	mp, °C	anal.	$[\alpha]^{19}_D$
1	2	H	A	72	153	C,H,N	-
2	3	H	A	69(a)	153	C,H,N, Cl	-
3	6	H	A	53 (a)	202	C,H,N, Cl	-
4	2	Me	A	41 (a)	109	C,H,N	D,L
5	6	Me	A	47 (a)	204	C,H,N	D,L
6	2	i-Bu	A	36	211	C,H,N	-24,4(b)
7	2	i-Bu	A	37	209	C,H,N	26(c)

(a) as dihydrochloride (b) D enantiomer, c 0.3, 6N HCI
(c) L enantiomer, c 0.32, 6N HCI
(d) C,H,N indicate that the experimental fits the calculated analysis within 0.3% error.

Table II. Data for compounds of structure Vb



(Vb)

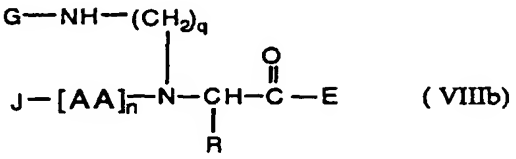
serial no.	q=	R	E	G	L	method	% yield	k'(a)	anal.	$[\alpha]_D^{19}$ (b)
8	2	H	OH	Z	H	B	37 (c)	-	C, H, N, Cl	-
9	2	H	OMe	Z	H	B, C	48	1.02	C, H, N	-
10	3	H	OMe	Z	H	B, C	34	1.08	C, H, N	-
11	6	H	OMe	Z	H	B, C	23	1.15	C, H, N	-
12	2	Me	OMe	Z	H	B, C	19	1.15	C, H, N	D, L
13	6	Me	OMe	Z	H	B, C	28	1.23	C, H, N	D, L
14	2	i-Bu	OMe	Z	H	B, C	31 (d)	1.26	C, H, N	7.27 (e)
15	2	i-Bu	OMe	Z	H	B, C	29	1.3	C, H, N	-7.1 (f)
16	3	H	OH	Boc	Fmoc	D	33	1.12	C, H, N	-
17	6	H	OH	Boc	Fmoc	D	26	1.26	C, H, N	-
18	3	i-Bu	OH	Z	Boc	E	43	-	C, H, N	-5.8
19	2	H	OH	Fmoc	Boc	E	42	1.08	C, H, N	-
20	2	H	OH	Fmoc	Boc	B	85	1.08	C, H, N	-
21	3	i-Bu	OH	Fmoc	Boc	E	94	1.3	C, H, N	-7.3

(a) RP-18 column, 70% MeOH (b) c 1, MeOH (c) as hydrochloride (d) include 1/3 H_2O
(e) D enantiomer (f) L enantiomer

Table III. ^{1}H NMR data on compounds 9 - 21

Compound	^{1}H NMR
9	7.4 (5H, Ar), 5.6 (1H, Amide), 5.08 (2H, Bz), 3.7 (3H, s,O-CH_3), 3.4 (2H, s, N-CH_2-CO), 3.25 (2H, m, CH_2), 2.75 (2H, t, CH_2), 1.8 (1H, s, Amine).
10	7.35 (5H, Ar), 5.75 (1H, Amide), 5.08 (2H, s, Bz), 3.07 (3H, s,O-CH_3), 3.35 (2H, s, N-CH_2-CO), 3.25 (2H, m, N-CH_2), 2.6 (2H, t, N-CH_2), 1.8 (1H, s, Amine).
11	7.35 (5H, Ar), 5.35 (1H, Amide), 5.08 (2H, s, Bz), 3.7 (3H, s,O-CH_3), 3.35 (2H, s, N-CH_2-CO), 3.15 (2H, m, N-CH_2), 2.5 (2H, m, N-CH_2), 1.85 (1H, s, Amine), 1..45 (4H, m, N-CH_2-**CH_2** -CH_2-CH_2-**CH_2** -CH_2-N), 1..3 (4H, m, CH_2-CH_2).
12	7.35 (5H, Ar), 5.4 (1H, Amide), 5.1 (2H, Bz), 3.7 (3H, s,O-CH_3), 3.35 (2H, m, N-CH_2), 3.22 (1H, m, αH), 2.6-2.8 (2H, m, N-CH_2), 1.85 (1H, s, Amine), 1.28 (3H, d, CH_3) .
13	7.35 (5H, Ar), 5.3 (1H, Amide), 5.1 (2H, s, Bz), 3.7 (3H, s,O-CH_3), 3.35 (1H, m, αH), 3.15 (2H, q, N-CH_2), 2.5 (2H, m, N-CH_2), 1.85 (1H, s, Amine), 1.5 (4H, m, N-CH_2-**CH_2** -CH_2-CH_2-**CH_2**-CH_2-N), 1.28 (7H, m, CH_3,CH_2-CH_2).
14, 15	7.35 (5H, Ar), 5.5 (1H, Amide), 5.1 (2H, s, Bz), 3.7 (3H, s,O-CH_3), 3.35 (2H, m, N-CH_2), 3.20 (H, m, αH), 2.7 (2H, m, N-CH_2), 1.9 (1H, s, Amine), 1.49 (2H, m, CH_2), 1.45 (1H, m, CH_2-**CH**(CH_3)$_2$), 0.9 (6H,d, CH_2-CH(**CH_3**)$_2$).
16	7.7 (2H, Ar), 7.6 (2H, Ar), 7.2-7.4 (4H, Ar), 4.4-4.7 (2H, m, Bz), 4.2 (1H, m, Fluorenyl-**CH**) , 3.8 (2H, m, CH_2), 2.7-3.5 (4H, m, N-**CH_2** -**CH_2** -**CH_2** -N), 1.45 (11H, s, CH_2+Boc).
17	7.7 (2H, Ar), 7.6 (2H, Ar), 7.2-7.4 (4H, Ar), 3-4.6 (9H, Fluorenyl-**CH_2**, CH_2CO, 2xCH_2-N, Bz), 1-1.5 (13H, s, CH_2-CH_2+Boc).
18	7.45 (5H, Ar), 6.0 (1H, NH), 5.2 (2H, m, Bz), 3.3-3.9 (5H, αH, **CH_2** -CH_2-**CH_2**) , 1.6-2.0 (5H, CH-CH_2, CH_2-**CH_2** -CH_2), 1.43 (9H, Boc).
19, 20	7.7 (2H, Ar), 7.6 (2H, Ar), 7.2-7.4 (4H, Ar), 4.4-4.7 (2H, m, Bz), 4.2 (1H, m, Fluorenyl-**CH**), 3.8 (2H, m, N-CH_2) , 2.7-3.5 (4H, m, CH_2-CH_2) , 1.45(9H, Boc).
21	7.8 (2H, Ar), 7.6 (2H, Ar), 7.2-7.4 (4H, Ar), 4.1-4.4 (4H, CHCO, CH_2, 3-3.4 (4H, 2xCH_2-N), 1.5-2 (4H, 2x CH_2)), 1.45(9H, Boc), 0.9 (6H,d, CH_2-CH(**CH_3**)$_2$).

Table IV. Data for compounds of structure VIII b [E=OMe; n=1; G=Z; J=Boc]



(VIIIb)

serial no.	AA	R	q=	method	% yield	[MH]$^+$ calc.	[MH]$^+$ found	k'(a)	anal.	$[\alpha]_D^{19}$ (b)
22	Phe	H	2	F	87	514	514	1.35	C, H, N	-3.43
23	Leu	H	2	F	98	480	480	1.48	C, H, N	-17.56
24	Trp(For)	H	2	F	98	581	581	1.6	C, H, N	-8.73
25	Phe	Me	2	G	98	528	528	2.16 (c)	C, H, N	4.43
26	Phe	i-Bu	2	F	95	570	570	2.1 (c)	C, H ,N	3.4
27	Phe	H	3	F	97	528	528	1.63	C, H, N	0.0
28	Phe	Me	6	G	99	584	584	2.4 (c)	C, H, N	-4.52
29	Phe	H	6	G	96	570	570	1.82	C, H, N	-4.58

PEPTIDE SYNTHESIS

[0054] A preferred method for the preparation of the peptides of this invention is according to the Solid Phase Peptide Synthesis methodology using a combination of Boc and Fmoc chemistries [e.g. Bodanszky, "Principles of Peptide Synthesis", Springer-Verlag, 1984; or Bodanszky *et al.*, "the Practice of Peptide Synthesis", Springer-Verlag, 1984; or Barany and Merrifield, in "The Peptides: Analysis, Synthesis and Biology", Vol. 2, Chapter 1, Academic Press, 1980; or Atherton *et al.*, Bioorg. Chem., 8, 1979). In the process of the present invention a preferred p-methyl benzhydrilamine polystyrene 1% divinyl benzene polymer (MBHA resin, degree of substitution was 0.9 eq. NH_2/gr., 1 gr (0.9 mmol)) was coupled with 2.4 mmol Boc-Met. The coupling was performed with BOP (2.4 mmol) and DIEA (5.6 mmol) in DMF. After 3 h the resin was reacted with Ac_2O (8 mmol) and DMAP (0.5 mmol) for 3 h and the resin washed 5 times with DCM.

Method M Boc Solid Phase Synthesis

[0055] Each synthetic cycle consisted of (i) a 1 min, followed by 20 min deprotection with TFA:DCM (1:2) (ii) 5 DCM washes (iii) 2X5 min neutralisation with 5% DIEA in DMF (iv) 5 DMF washes (v) 60 min coupling with 2.4 mmol Boc-AA using BOP (2.4 mmol) and DIEA (5.6 mmol) in DMF (vi) checking for completion by the Kaiser test [Kaiser, E., *et al.*, Anal. Biochem. 34, 595 (1970)] (vii) 5 DCM washes.

Method N Fmoc Solid Phase Synthesis

[0056] Each synthetic cycle consisted of (i) a 17 min deprotection with 20% piperidine in DMF (ii) 6 DMF washes (iii) 60 min coupling with 2.4 mmol Fmoc-AA using BOP (2.4 mmol) and DIEA (5.6 mmol) in DMF (iv) 6 DMF washes, and (v) Kaiser test.

[0057] Side-chain protection: Arg(Tos); Asp(OBzl); N(mercaptopropylene)Gly(SBzl); 5-mercapto valeric acid(SBzl);

N(g-Boc aminopropylene)Gly.

Method P Removal of the Peptide from the Resin and Subsequent Removal of Protecting Groups

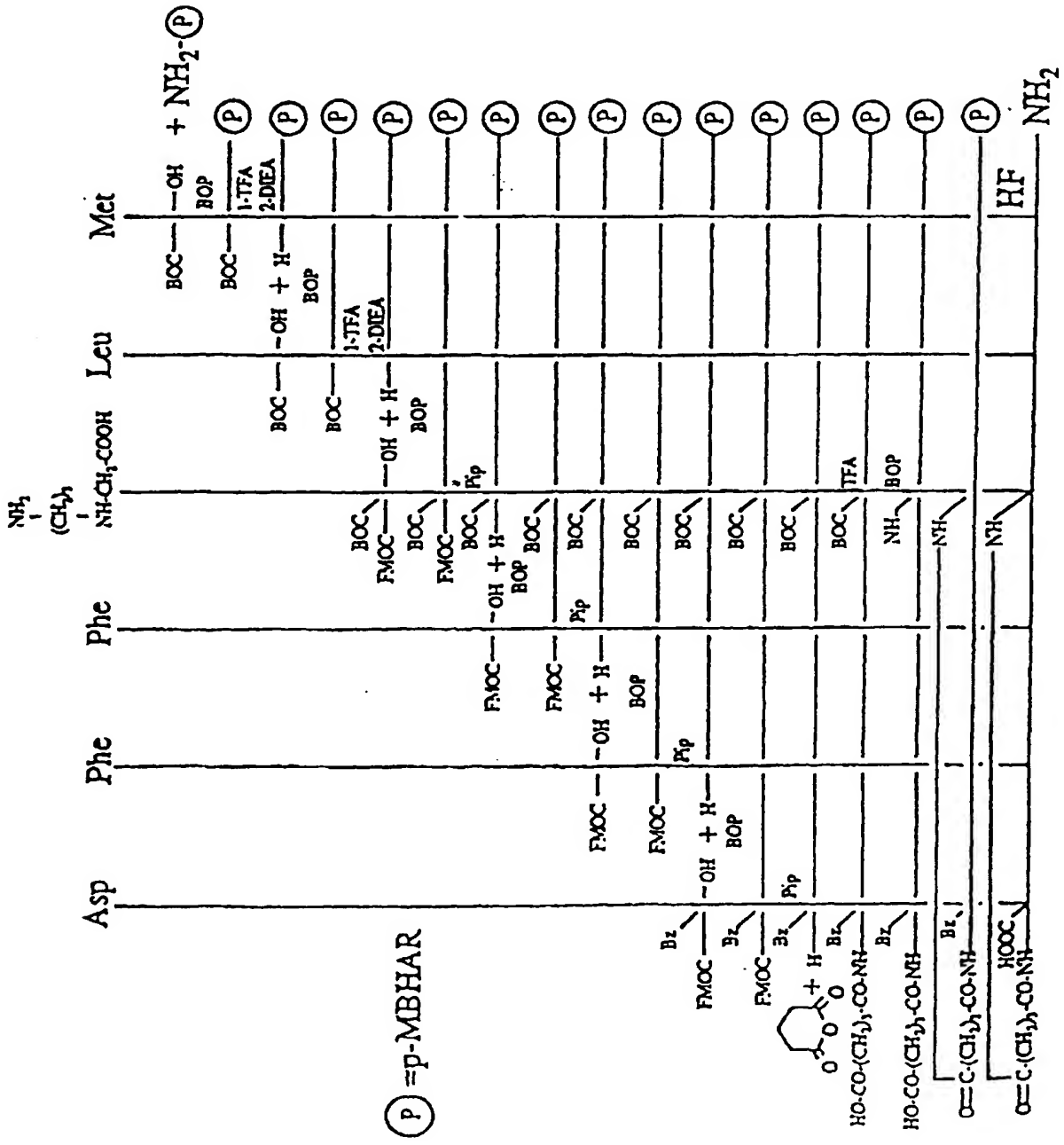
[0058] In a Kel-F vessel were put: 1 gr resin, magnetic stirrer and 2 mL anisole. The vessel was attached to the a rector [Type I made by the Peptide Institute Inc. Osaka, Japan].. After removal of air by vacuum, the reaction vessel was frozen by liquid nitrogen and charged with 20 ml liquid HF. The vessel was kept for 1.5 h at -7°C with stirring. HF was evaporated to dryness and hexane (50 mL) was added to the reaction vessel. After mixing, the hexane was decanted and the reaction mixture was washed with ether (3X50 mL) which was decanted. The mixture was treated with 30% AcOH (3X50 mL) and filtered on a sintered glass funnel. The solvent was lyophilized. The yields of the crude peptides was 50 - 80%.

Method Q on Resin Cyclization of Peptides Ia-Ic

[0059] After the main chain was synthesized, the Boc protecting group was removed from N(g-aminopropylene)Gly by TFA and the resin washed and neutralized as described for Boc Solid Phase Synthesis (i) - (iv). Cyclization [Felix, A. M., *et al*., in "Peptides" Marshall, G. B., Ed. p. 465 (1988)] was performed with BOP (2.4 mmol) an DIEA (3.6 mmol) for 24 h. After 24 h, if Kaiser test was negative, the resin was washed with MeOH and dried in vacuo over P_2O_5. If the Kaiser test was positive, another portion of BOP (1.2 mmol) and DIEA (1.8 mmol) were added. After the cyclization peptides were removed from the resin as described above.

Synthesis of Peptide Ic

[0060] Peptide Ic was synthesized according to the following scheme using general methods M, N, P and Q.



The crude peptide was purified on semiprep HPLC on HIBAR RP-8 column with gradient of A=H_20 (0.1% TFA); B=MeCN (0.1% TFA). t=0, A=70, B=30; t=10, A=70, B=30; t=50, A=20, B=80, t=60, A=0, B=100; R_t=20.3 min. MW=880.06; FAB-MS found $[MH]^+$=881.14; API-MS found $[MH]^+$=881.60; AAA found Asp=0.9; Phe=1.95; Met=0.95; Leu=1.0.

Synthesis of Peptide Ia

[0061] Peptide was synthesized according to the scheme of peptide Ic above using general methods M, N, P and Q. Instead of Fmoc-Asp(OBzl), Fmoc-Arg(Tos) was coupled and instead of glutaric anhydride the peptide was reacted with adipic acid.

The crude peptide was purified on semiprep HPLC on HIBAR RP-8 column with gradient of A=H_20 (0.1% TFA);

B=MeCN (0.1% TFA). t=0, A=70, B=30; t=10, A=70, B=30; t=50, A=20, B=80, t=60, A=0, B=100; R_t=20.3 min. MW=880.06; FAB-MS found $[MH]^+$=881.14; API-MS found $[MH]^+$=881.60; AAA found Asp=0.9; Phe=1.95; Met=0.95; Leu=1.0.

Synthesis of Peptide Ib

[0062] Peptide Ib was synthesized according to the scheme of peptide Ic above using general methods M, N, P and Q. Instead of Fmoc-Asp(OBzl), Fmoc-Arg(Tos) was coupled.
The crude peptide was purified on semiprep HPLC on HIBAR RP-8 column with gradient of A=$H_2$0 (0.1% TFA); B=MeCN (0.1% TFA). t=0, A=60, B=40; t=10, A=60, B=40; t=40, A=20, B=80; t=50, A=0, B=100; R_t=24 min. MW=962.25; FAB-MS found $[MH]^+$=963.1; API-MS found $[MH]^+$=963.2; AAA found Arg=0.9; Phe= 2.05; Met=0.85; Leu=0.95. Proton NMR peak assignment is shown in Table V.

Synthesis of Peptide Id

[0063] Peptide Id was synthesized according to the scheme of peptide Ic above using general methods M, N, P and Q. Instead of Fmoc-Asp(OBzl), Fmoc-Arg(Tos) was coupled. Instead of Fmoc-N(g-Boc-amino propylene)Gly, Fmoc-N(g-mercapto(SBzl) propylene)Gly was used and instead of glutaric anhydride, d-mercapto(SBzl)valeric acid was used.

Cyclization of Peptide Id

[0064] Peptide Id was removed from the resin by HF to yield the reduced form. After lyophilization the crude peptide dissolved in MeOH (1 L) and I_2 (0.8 mmol) was added. The solution kept at room temperature for 72 h with stirring. The advance of the oxidation reaction was followed by HPLC. Residual SH groups were determined by the Ellman test (Ellman, G. L., Biochem. Biophys. 82, 70 (1959)]. After completion, the solvent was evaporated in vacuo and the peptide purified by preparative HPLC.

Table V. Proton chemical shifts of peptide Ib in DMSO at 303 K.[a]

Residue	NH	$C^{\alpha}H$	$C^{\beta}H$	$C^{\gamma}H$	$C^{\delta}H$	other
Arg^1	8.13	3.92	1.58 1.44	1.33 1.28	3.01	$C^{\epsilon}H$ 7.47
	(7.87)	(4.01)	(1.54) (1.40)	(1.31) (1.20)	(2.98)	(7.44)
Phe^2	7.08	4.55	3.02 R 2.77 S			aromatic 7.12-7.29
	(7.34)	(4.41)	(3.08 S) (2.82 R)			(7.12-7.29)
Phe^3	9.02	4.92	3.04 S 2.79 R			aromatic 7.12-7.29
	(8.35)	(4.58)	(3.16 S) (2.80 R)			(7.12-7.29)
$NGly^4$		3.99 3.76				
		(4.51) (3.91)				
Leu^5	8.01	4.26	1.48 1.43	1.65	0.89 0.85	
	(8.43)	(4 37)	(1.47) (1.43)	(1.63)	(0.89) (0.88)	
Met^6	7.92	4.24	1.94 1.83	2.46 2.37		$C^{\epsilon}H_3$ 2.01
	(8.08)	(4.23)	(1.80) (1.72)	(2.38) (2.28)		(2.01)
I		2.28 2.03	1.76 1.73	2.08 1.97		
		(2 23) (2.08)	(1.78) (1.70)	(2.08) (1.98)		
II	7.56	2.97	1.52 1.45	3.33		
	(7.31)	(3.26) (2.91)	(1.71) (1.62)	(3.82) (2.85)		
NH_2	7.24,7.04 (7.16,7.01)					

[a]The chemical shifts of the minor isomer (accounting for 18%) with a cis configuration of the Phe^3-$NGly^4$ peptide bond are given in parentheses. The prochiral assignments of the β-protons of the Phe residues is given.

Synthesis of Peptide IIa

[0065] Peptide IIa was synthesized according to the scheme of peptide Ic above using general methods M, N, P and

Q. Instead of Boc-Met, Boc-Hcys(SBzl) was coupled to the PMBHA resin. Instead of Fmoc-Asp(OBzl), Fmoc-Arg(Tos) was coupled and instead of glutaric anhydride the peptide was reacted with acetic anhydride.

Cyclization of Peptide IIa

[0066] Peptide IIa was removed from the resin in the reduced form. After lyophilization the crude peptide dissolved in a saturated NH_3/MeOH solution (1 L) at 0°C. The solution kept at room temperature for 72 h with stirring. The advance of the reaction was followed by HPLC. Residual SH groups were determined by the Ellman test [Ellman, G. L., Biochem. Biophys. 82, 70 (1959)]. After completion, the solvent was evaporated in vacuo and the peptide purified by semi-preparative HPLC on RP-18 HIBAR column with gradient of A=H_2O (0.1% TFA); B=MeCN (0.1% TFA). t=0, A=60, B=40; t=10, A=60, B=40; t=40, A=20, B=80; t=50, A=0, B=100; R_t=25 min.
MW=893.7; FAB-MS found $[MH]^+$=894.9; API-MS found $[MH]^+$=894.8; AAA found Arg=0.9; Phe= 1.95; Hcys=0.75; Leu=1.0

Synthesis of Peptide IIIb

[0067] Peptide IIIb was synthesized according to the following scheme using general methods M, N and P. The crude peptide was purified on semiprep HPLC on HIBAR RP-8 column with gradient of A=H_2O (0.1% TFA); B=MeCN (0.1% TFA). t=0, A=70, B=30; t=5, A=70, B=30; t=40, A=20, B=80; t=50, A=0, B=100; R_t=22,7 min.MW=995.3; FAB-MS found $[M]^+$=995.4; API-MS found $[MH]^+$=996.0; AAA found Arg=1.0; Phe= 2.0; Met=0.8; Leu=0.95.

Synthesis of Peptide IIIa

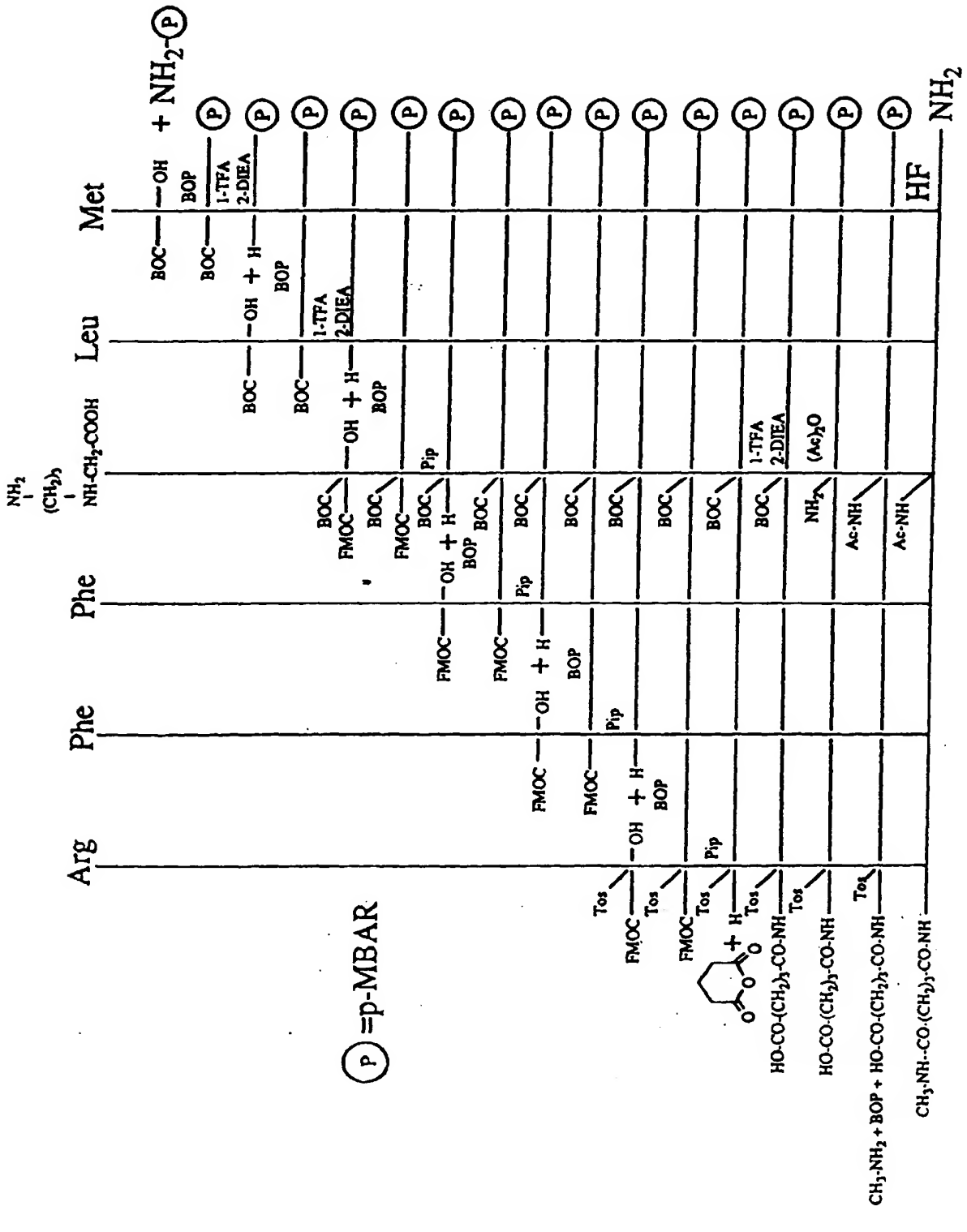
[0068] Peptide IIIa was synthesized according to the scheme of peptide IIIb above using general methods M, N and P. Instead of glutaric anhydride the peptide was reacted with acetic anhydride. After acetylation the peptide was removed from the resin by HF.
The crude peptide was purified on semiprep HPLC on HIBAR RP-18 column with gradient of A=H_2O (0.1% TFA); B=MeOH (0.1% TFA). t=0, A=60, B=40; t=30, A=0, B=100; R_t=24 min.
MW=854.5; FAB-MS found $[MH]^+$=855.6; API-MS found $[MH]^+$=855.6; AAA found Arg=0.8; Phe= 1.95; Met=0.85; Leu=0.95

BIOLOGICAL ACTIVITY EXAMPLES

General

[0069] The biological activity of the cyclic (and comparative linear) peptides was assayed in different smooth muscle systems: Vas Deferens Rat (RVD), Guinea Pig Ileum (GPI) and Rat Portal Vein (RPV).

[0070] Assay of the activity of the different analogs in these systems enables the determination of their selectivity towards tachykinin receptors present in these tissues, namely NK-1 and NK-3 receptors in GPI, NK-3 in RPV and NK-2 in RVD. The presence of two tachykinin receptors in the GPI system prevents direct and selective assay of each one of them separately, and therefore one receptor had to be blocked when assaying the activity of the analog towards the second receptor. Blocking the NK-3 receptor in the GPI system by blocking the acetylcholine receptors with atropine enabled assaying the NK-1 receptor separately.


Met
Leu
Phe
Phe
Arg
NH2-(CH2)3-NH-CH2-COOH
BOC
FMOC
OH + H
BOP
1-TFA
2-DIEA
Pip
(Ac)2O
Ac-NH
Tos
HF
NH2
P =p-MBAR
HO-CO-(CH2)3-CO-NH
CH3-NH2 + BOP + HO-CO-(CH2)3-CO-NH
CH3-NH-CO-(CH2)3-CO-NH

Methods

[0071] Assay of the resistance of the analog peptides to digestion by proteases was performed by incubating the peptide with slices, homogenates or membranes of various tissues such as brain, liver, kidney, parotid gland, etc., and assaying the residual activity after incubation, by the GPI assay.

(a) Guinea Pig Ileum Assay (GPI)

[0072] Assay was performed according to the procedures described in Wormser, U., *et al* ., EMBO J. 5, 2805 (1986).

(b) Rat Vas Deferens Assay (RVD)

[0073] Assay was performed according to the procedure described in Chorev *et al* ., Eur. J. Pharmacol., 127, 187 (1986).

(c) Rat Portal Vein Assay (RPV)

[0074] Rat was sacrificed by decapitation. The abdomen was cut open and all internal organ moved to the side. The portal vein was tied at both ends while within the animal and cleaned from all surrounding tissues, to obtain a clean tissue. The cleaned tissue was immersed in a bath containing Tyrode solution, aerated with a mixture of CO_2:O_2(95:5%). One of the tied ends was attached to a glass hook and the other end to a transducer lever, in order to measure the contractions. Tension was about 0.5g. The tissue was left in the bath at 37°C for an hour, then the peptides were added at 20 min intervals, to prevent desensitization of the receptor.

(d) Resistance to digestion by proteases

[0075] Assay was performed according to the procedures described in Chorev *et al* ., Eur. J. Pharmacol., 127, 187 (1986).

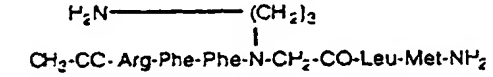
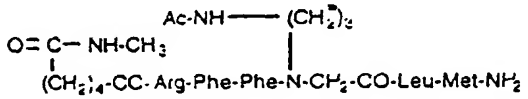
[0076] The following specific examples demonstrate the activity of the peptides Ia, Ib, Ic, Id and IIa, some in comparison with the linear, prior art WS septide.

A. Biological activity of cyclized peptides

[0077]

	Peptide		Biological
Activity [EC_{50}(nM)]			
		NK-1	NK-2
NK-3			
>100,000	Ib	11	>50,000
>100,000	Ia	5	>50,000
>50,000	Ic	15	>100,000
>100,000	IIa	20	>100,000
>100,000	WS septide	3	>200,000

[0078] All of the analogs were highly selective to the NK-1 sub-receptor, as the prior art linear analog WS Septide. Figure 3 shows the dose response curve of WS septide and peptide Ia.
In order to prove that the high biological activity and selectivity indeed resulted from the cyclization of the peptides, linear analogs IIIa and IIIb were prepared. Analog IIIa contains N-(b-aminoethyl)glycine^{9} (9th position of Substance P) , the b-amine function being free. Analog IIIb is the nearest linear imitation of peptide Ia, containing the groups CH_3-NH-Glur-Arg^{6} (6th position of Substance P) and Ac-NH-[$(CH_2)_3$]-Gly^{9} (9th position of Substance P). In order to avoid electrical charge influence on the activity, the N-terminus was blocked by acylation and the glutaric end by N-methylation. The low activity and lack of selectivity of the linear peptides derived from peptide Ia prove the importance of the cyclization in achieving the conformational restriction which is required for increased activity and selectivity.

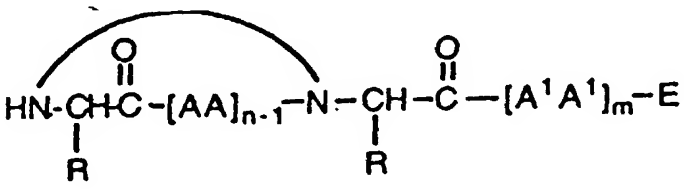
	PEPTIDE STRUCTURE	RECEPTOR SUBTYPES [IC_{50} (nM)]		
		NK-1	NK-2	NK
IIIa		300	>100,000	2000
IIIb		400	>100,000	2000

B. Resistance to Proteases

[0079] Figure 2 shows the degradation of WS septide (linear) and peptide Ia in parotid tissue. While the linear WS septide is highly active and selective towards the NK-1 receptor, it is metabolically unstable and activity was lost already after a few minutes (half life = 6 min). Peptide Ia retains a 80% activity even after 120 min. of incubation with parotid slices.
[0080] The same pattern of behavior can be seen with incubation with liver slices. 50% of the biological activity of the peptide Ia were conserved after 30 min of incubation with the tissue.

Claims

1. A process for the preparation of cyclic biologically active polypeptides of the general formula (I),



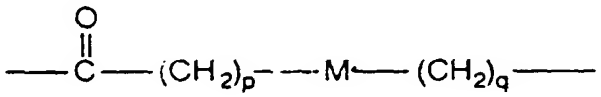
(I)

wherein

n designates an integer of from 1 to 10;
m designates an integer of from 1 to 10;

[AA] designates a naturally occurring or synthetic amino acid residue, wherein the amino acid residues in case n is greater than 1 may be the same or different;
[A^1 A^1] designates a naturally occurring or synthetic amino acid residue, wherein the amino acid residues in case m is greater than 1 may be the same or different;
R and R' designate a naturally occurring or synthetic amino acid side-chain;
E represents a hydroxyl, or a carboxyl protecting group which is standard in peptide synthesis selected from alkoxy, substituted alkoxy or aryloxy, or a blocking group which may be the same as the carboxyl group or an

amino or substituted amino group, wherein the carboxyl protecting or blocking group may be optionally covalently attached to an insoluble polymeric support, and the circled line designates a spacer group of the formula:

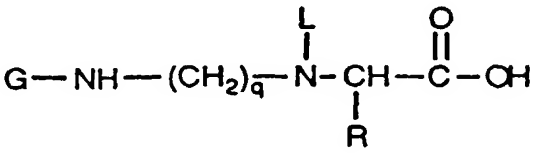


(III)

wherein M is selected from the group consisting of -S-S-, -CO-NH- and -S- and p and q, which may be the same or different, each designates an integer of from 2 to 10;

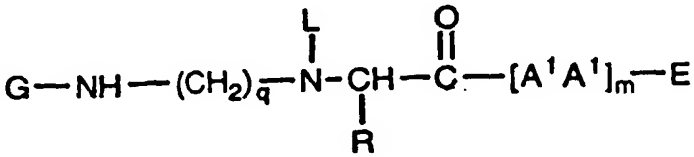
which process comprises the steps of:

(a) reacting a compound of the formula:



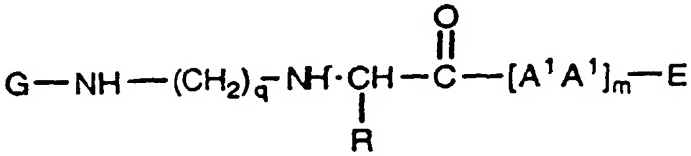
(Va)

wherein q represents an integer of from 2 to 10, G and L, which may be the same or different, each represents a protecting group conventional in peptide synthesis, with an amino acid or a polypeptide of the formula H_2N-$[AA]_m$-CO-E, wherein m is an integer from 1 to 10 and AA represents a naturally occurring or synthetic amino acid residue, the amino acid residues in case m is greater than 1 being the same or different, and E represents a carboxyl protecting group selected from alkoxy, substituted alkoxy or aryloxy, or a blocking group which may be the same or an amine or substituted amine group, wherein the carboxyl protecting or blocking group may be optionally covalently attached to an insoluble polymeric support to give a compound of the formula:



(VIa)

b) selectively removing from the compound of formula VIa, protecting group L, to give a compound of the formula:

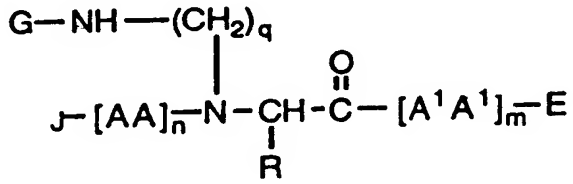


(VIIa)

c) reacting the compound of formula VIIa with an amino acid or peptide of the formula:

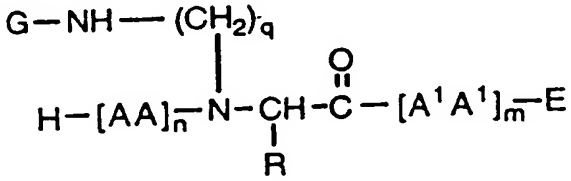
$$J—[AA]_n—OH \qquad (VIIb)$$

wherein n is an integer of from 1 to 10, [AA] represents a naturally occurring or synthetic amino acid residue, the amino acid residues in case n is greater than 1 being the same or different and J represents a protecting group conventional in peptide synthesis, to give a compound of the formula:



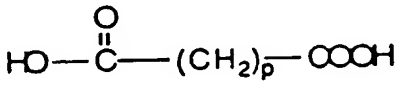
(IXa)

d) selectively removing the protecting group J to give a compound of the formula:



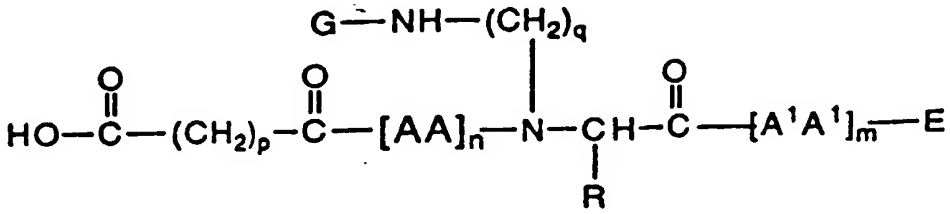
(Xa)

e) reacting the compound of formula Xa with a compound of the formula:



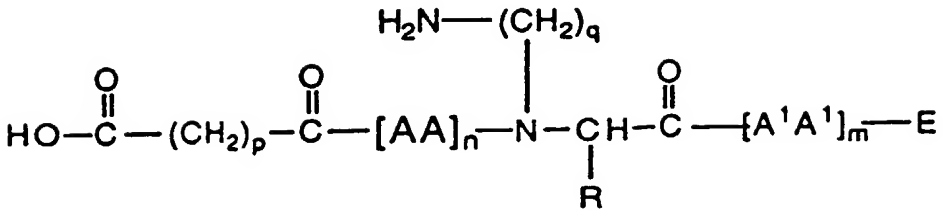
(XIa)

wherein p is as defined above, to give a compound of the formula :



(XIIa)

f) selectively removing from the compound XIIa the protecting group G to give a compound of the formula:



(XIIIa)

g) reacting the compound of formula XIIIa with a suitable coupling agent selected from the group consisting of dicyclohexylcarbodiimide (DCC), bis(2-oxo-3-oxazolidinyl) phosphinic chloride (BOP-Cl), benzotriazolyl-N-oxytrisdimethylaminophosphonium hexafluoro phosphate (BOP), 1-oxo-1-chlorophospholane (Cpt-Cl), and a mixture of DCC and hydroxybenzotriazole (HOBT) and removing other side-chain protecting groups to give a compound of the general formula I where m is an integer from 1 to 10.

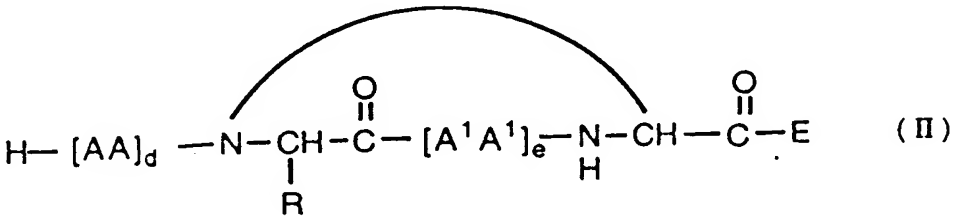
2. A process according to claim 1 wherein the step (a) G is selected from the group consisting of Z, Boc and Fmoc.

3. A process according to claim 1 wherein in step (a) L is selected from the group consisting of Z, Boc and Fmoc.

4. A process according to claims 2 and 3 wherein G is Boc and L is Fmoc.

5. A process according to claims 2 and 3 wherein G is Fmoc and L is Boc.

6. A process for the preparation of biologically active cyclized polypeptides of general formula (II)



(II)

wherein

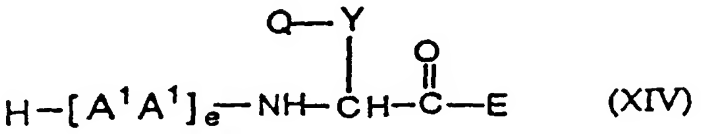
d is 0 or an integer of from 1 to 10 and e is an integer of from 1 to 10;
[AA] designates a naturally occurring or synthetic amino acid residue, wherein the amino acid residues in case e is greater than 1 may be the same or different;
[A^1 A^1] designates a naturally occurring or synthetic amino acid residue, wherein the amino acid residues in case d is greater than 1 may be the same or different;
R designates a naturally occurring or synthetic amino acid side-chain;
E represents a hydroxyl, or a carboxyl protecting group which is standard in peptide synthesis, preferably selected from alkoxy, substituted alkoxy or aryloxy, or a blocking group which may be the same as the carboxyl group or an amino or substituted amino group, wherein the carboxyl protecting or blocking group may be optionally covalently attached to an insoluble polymeric support, and the circled line designates a spacer group of the formula:

$$—(CH_2)_p—(M)_x—Y \qquad (IV)$$

wherein p is as defined above and M is an amino or a carboxyl group or a sulfur atom, x is 0 or 1, and Y des-

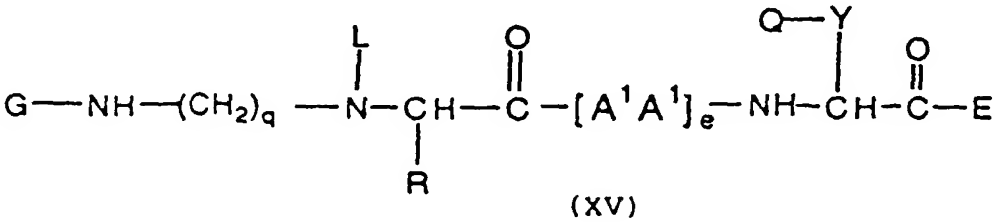
ignates the side-chain of a backbone amino acid which process comprises the steps of:

a) reacting a compound of the formula:



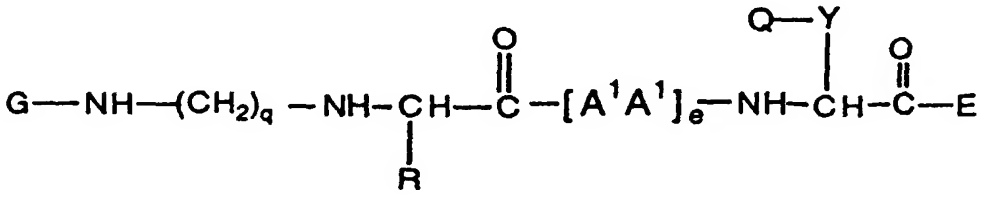
(XIV)

wherein E and e are as defined above, $[A^1A^1]$ represent a naturally occurring or synthetic amino acid residue and Y represents a side-chain of naturally occurring or synthetic amino acid and Q represents a protecting group conventional for peptide synthesis with a compound of formula Va, according to claim 1 to give a compound of the formula:



(XV)

b) selectively removing from the compound of formula XV protecting group L, to give a compound of the formula:

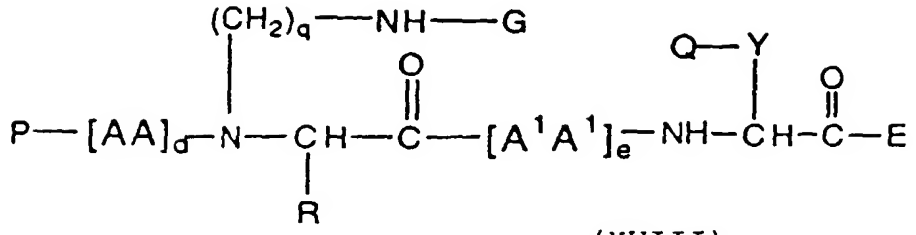


(XVI)

c) reacting the compound of formula XVI with an amino acid or peptide of the formula:

$$P—[AA]_d—OH \qquad (XVII)$$

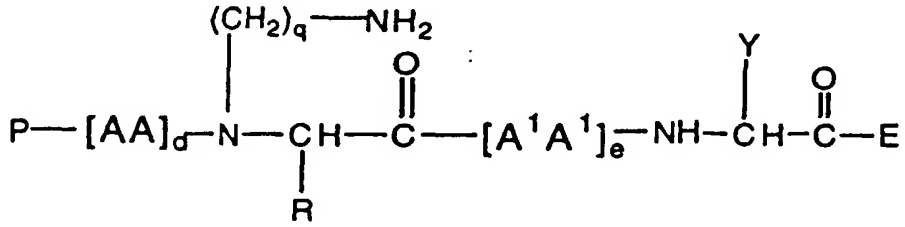
wherein d is an integer of from 1 to 10 and [AA] represents a naturally occurring or synthetic amino acid residue, the amino acid residues in case d is greater than 1 being the same or different, and P represents a protecting group conventional in peptide synthesis, to give a compound of the formula:



(XVIII)

d)

(i) in case Y is a carboxyl bearing side-chain, selectively removing the protecting groups G and Q from compound XVIII to give a compound of the formula:



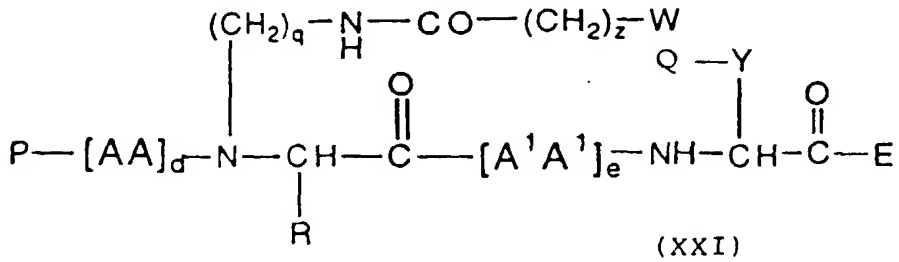
(XIX)

cyclizing the compound XIX by reacting it with the coupling agent according to step g) of claim 1 and removing protecting group P and other side-chain protecting groups to give a compound of the general formula II; or

(ii) in case Y is a side-chain bearing a nucleophilic functional group other than carboxyl, selectively removing protecting group G, reacting the resulting compound with a compound of the formula:

$$HOOC—(CH_2)_z—W \qquad (XX)$$

wherein z is an integer of from 1 to 10 and W is a function bearing group capable of reacting with said nucleophilic group to give a compound of the formula:



(XXI)

selectively removing the protecting group Q, wherein cyclization occurs, and removing the protecting group P and other side-chain protecting groups from compound XXI to give a compound of the general formula II.

7. A process according to claim 6 wherein Y represents the side-chain of homocysteine or cysteine, Q is benzyl and E is an amide.

8. A process according to claim 6 or claim 7 wherein G is selected from the group consisting of Z, Boc and Fmoc.

9. A process according to claim 6 or claim 7 wherein L is selected from the group consisting of Z, Boc and Fmoc.

10. A process according to claim 9 wherein G is Boc and L is Fmoc.

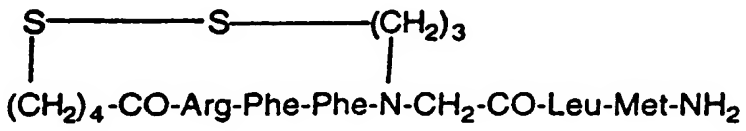
11. A process according to any one of claims 6 to 10, wherein in step (c) P is acetyl.

12. A process according to any one of claims 6 to 10, wherein E is an amide.

13. A process according to any one of claims 6 to 11, wherein in step (d) (ii) W is selected from the group consisting of halogen atoms, O-p-toluenesulphonyl, O-methanesulphonyl and O-trifluoromethanesulphonyl.

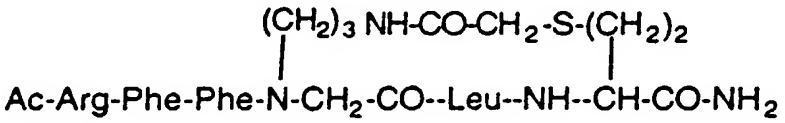
14. A cyclic biologically active polypeptide having the formula:

Id

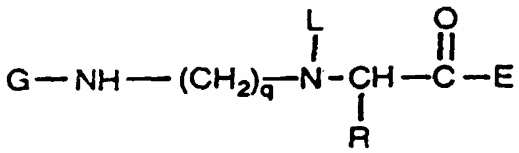


15. A cyclic biologically active polypeptide having the formula:

IIa



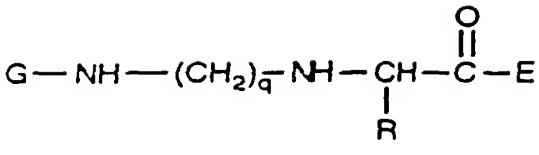
16. A process for the preparation of compounds of general formula Vb:



(Vb)

wherein q represents an integer of from 2 to 10, G and L, which may be the same or different, each represents hydrogen and/or a protecting group conventional in peptide synthesis, and E represents a hydroxyl, or a carboxyl protecting group which is standard in peptide synthesis, preferably selected from alkoxy, substituted alkoxy or aryloxy, or a blocking group which may be the same as the carboxyl group or an amino or substituted amino group, wherein the carboxyl protecting or blocking group may be optionally covalently attached to an insoluble polymeric support by;

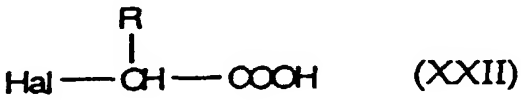
(i) reacting the free acid Va according to claim 1 with the appropriate reagent containing the group E to give the ester, amide or covalent bond with the polymeric support; or
(ii) from compounds of the formula VIb:



(VIb)

by reacting this free acid with the appropriate reagent containing the group E, to give the ester, amide or covalent bond with the polymeric support; compound of the formula VIb is obtained from compound XXVI according to claim 17 by reacting this free acid with the appropriate reagent containing the group E defined above, to give the ester, amide or covalent bond with the polymeric support.

17. A process for the preparation of compounds of general formula Va, according to claim 1 which process comprises (a) an α-halocarboxylic acid of the formula:

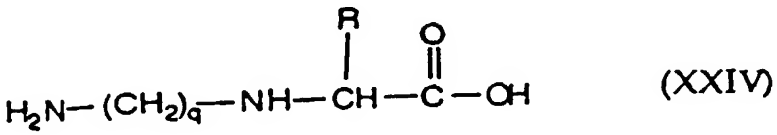


(XXII)

wherein R is as defined in claim 1 and Hal is chlorine, bromine or iodine, is reacted with an alkylene diamine of the formula:

$$H_2N—(CH_2)_q—NH_2 \qquad \text{(XXIII)}$$

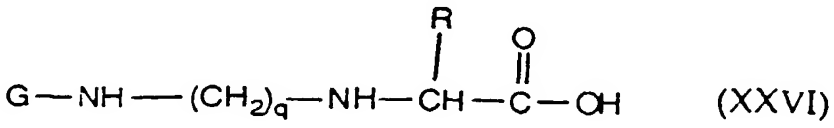
wherein q is as defined in claim 1, to give a compound of the formula:



(XXIV)

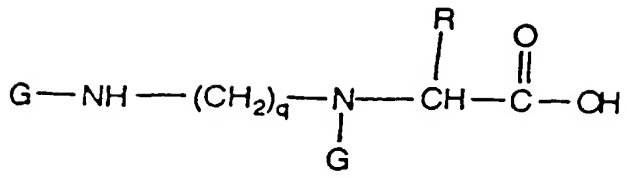
(b)

(i) reacting the compound of formula XXIV with suitable reagent containing the group G by standard methods of peptide synthesis, where G is according to claim 1, to give a compound of the formula:



(XXVI)

and reacting the compound of formula (XXVI) with a reagent containing L by standard methods of peptide synthesis, where L is defined in claim 1, to give a compound of the formula Va, or
(ii) reacting the compound of formula XXIV with a suitable reagent containing G by standard methods of peptide synthesis, where G is as defined above to give a compound of formula:



(XXVIII)

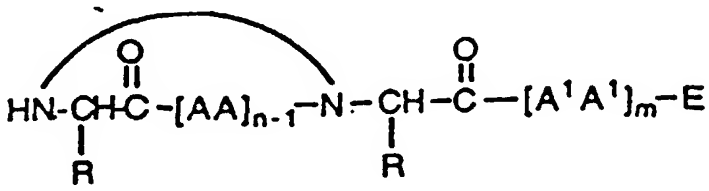
selectively removing the protecting group G from the secondary amino function to give a compound of the general formula XXVI.

18. A pharmaceutical composition, comprising the backbone cyclised polypeptide according to claims 14 or 15 which comprises any of compounds of formula, Id or IIa.

19. A pharmaceutical composition according to claim 18 for the treatment of pain, inflammation, Alzheimers disease, Familial Dysautonomia, Parkinson's Disease and Retardive Dyskinesia.

Patentansprüche

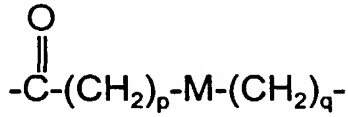
1. Verfahren zur Herstellung von cyclischen, biologisch aktiven Polypeptiden der allgemeinen Formel (I):



(I)

worin

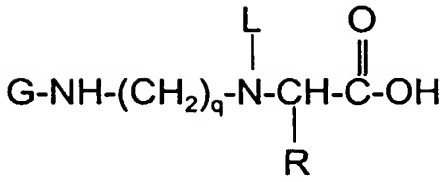
n eine ganze Zahl von 1 bis 10 darstellt,
m eine ganze Zahl von 1 bis 10 darstellt,
[AA] einen natürlich vorkommenden oder synthetischen Aminosäurerest darstellt, wobei die Aminosäurereste, wenn n größer 1 ist, gleich oder verschieden sein können,
[A^1 A^1] einen natürlich vorkommenden oder synthetischen Aminosäurerest darstellt, wobei die Aminosäurereste, wenn m größer 1 ist, gleich oder verschieden sein können,
R und R' eine natürlich vorkommende oder synthetische Aminosäure-Seitenkette darstellen,
E eine Hydroxylgruppe, oder eine Carboxylschutzgruppe, die in der Peptidsynthese Standard ist, ausgewählt aus Alkoxy, substituiertem Alkoxy oder Aryloxy, oder eine Blockiergruppe, die wie die Carboxylgruppe oder eine Amino- oder substituierte Aminogruppe sein kann, darstellt, wobei die Carboxylschutzgruppe oder Blokkiergruppe gegebenenfalls kovalent an einen unlöslichen Polymerträger gebunden sein kann, und die Bogenlinie eine Abstandhaltegruppe der Formel:



(III)

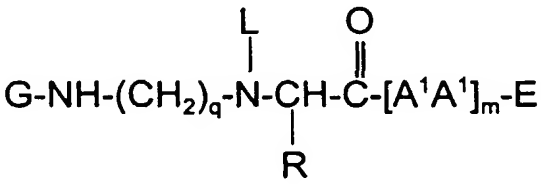
darstellt, worin M aus der Gruppe, bestehend aus -S-S-, -CO-NH- und -S-ausgewählt wird, und p und q, die gleich oder verschieden sein können, jeweils eine ganze Zahl von 2 bis 10 darstellen, wobei das Verfahren die Schritte umfaßt

(a) Umsetzen einer Verbindung der Formel:



(Va)

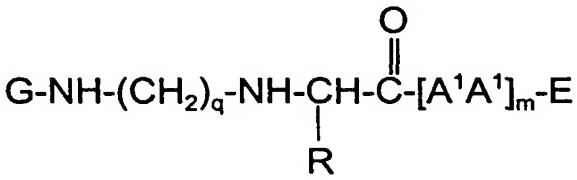
worin q eine ganze Zahl von 2 bis 10 darstellt, G und L, die gleich oder verschieden sein können, jeweils eine in der Peptidsynthese gebräuchliche Schutzgruppe darstellen, mit einer Aminosäure oder einem Polypeptid der Formel H_2N-$[AA]_m$-CO-E, worin m eine ganze Zahl von 1 bis 10 ist und AA einen natürlich vorkommenden oder synthetischen Aminosäurerest darstellt, wobei die Aminosäurereste, wenn m größer 1 ist, gleich oder verschieden sein können, und E eine Carboxylschutzgruppe, ausgewählt aus Alkoxy, substituiertem Alkoxy oder Aryloxy, oder eine Blockiergruppe, die die gleiche oder eine Amin- oder substituierte Amingruppe sein kann, darstellt, wobei die Carboxylschutzgruppe oder Blockiergruppe gegebenenfalls kovalent an einen unlöslichen Polymerträger gebunden sein kann, wobei eine Verbindung der Formel:



(VIa)

erhalten wird,

(b) selektives Entfernen der Schutzgruppe L von der Verbindung nach Formel VIa, wobei eine Verbindung der Formel:



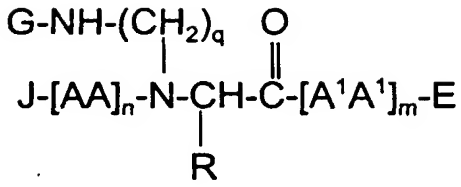
(VIIa)

erhalten wird,

(c) Umsetzen der Verbindung nach Formel VIIa mit einer Aminosäure oder einem Peptid der Formel:

$$J\text{-}[AA]_n\text{-}OH \qquad \text{(VIIb)}$$

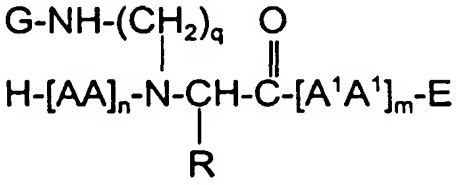
worin n eine ganze Zahl von 1 bis 10 ist, [AA] einen natürlich vorkommenden oder synthetischen Aminosäurerest darstellt, wobei die Aminosäurereste, wenn n größer 1 ist, gleich oder verschieden sein können, und J eine in der Peptidsynthese gebräuchliche Schutzgruppe darstellt, wobei eine Verbindung der Formel:



(IXa)

erhalten wird,

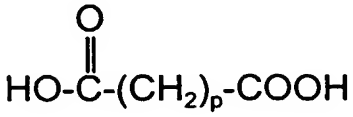
(d) selektives Entfernen der Schutzgruppe J, wobei eine Verbindung der Formel:



(Xa)

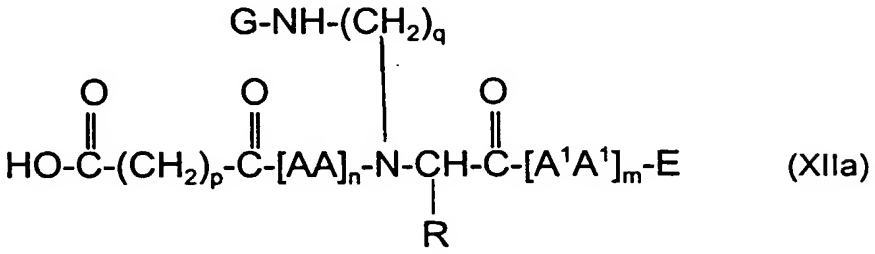
erhalten wird,

(e) Umsetzen der Verbindung nach Formel Xa mit einer Verbindung der Formel:



(XIa)

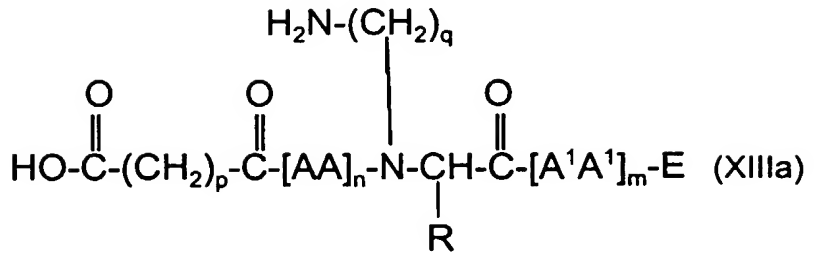
worin p wie vorstehend definiert ist, wobei eine Verbindung der Formel:



(XIIa)

erhalten wird,

(f) selektives Entfernen der Schutzgruppe G von der Verbindung XIIa, wobei eine Verbindung der Formel:



(XIIIa)

erhalten wird,

(g) Umsetzen der Verbindung nach Formel XIIIa mit einem geeigneten Kopplungsmittel, ausgewählt aus der Gruppe, bestehend aus Dicyclohexylcarbodiimid (DCC), Bis(2-oxo-3-oxazolidinyl)phosphinchlorid (BOP-Cl), Benzotriazolyl-N-oxytrisdimethylaminophosphoniumhexafluorophosphat (BOP), 1-Oxo-1-chlorphospholan (Cpt-Cl) und einem Gemisch aus DCC und Hydroxybenzotriazol (HOBT), und Entfernen anderer Seitenketten-Schutzgruppen, wobei eine Verbindung der allgemeinen Formel I, worin m eine ganze Zahl von 1 bis 10 ist, erhalten wird.

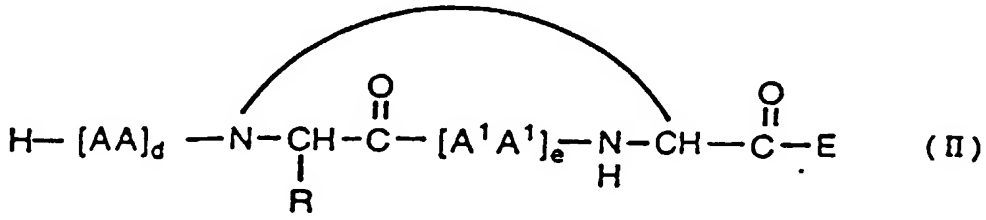
2. Verfahren nach Anspruch 1, wobei in Schritt (a) G aus der Gruppe, bestehend aus Z, Boc und Fmoc ausgewählt wird.

3. Verfahren nach Anspruch 1, wobei in Schritt (a) L aus der Gruppe, bestehend aus Z, Boc und Fmoc ausgewählt wird.

4. Verfahren nach den Ansprüchen 2 und 3, worin G Boc ist und L Fmoc ist.

5. Verfahren nach den Ansprüchen 2 und 3, worin G Fmoc ist und L Boc ist.

6. Verfahren zur Herstellung von biologisch aktiven cyclisierten Polypeptiden der allgemeinen Formel (II):



(II)

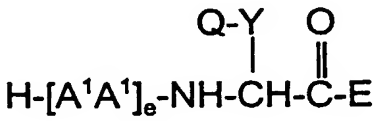
worin

d 0 oder eine ganze Zahl von 1 bis 10 ist und e eine ganze Zahl von 1 bis 10 ist,
[AA] einen natürlich vorkommenden oder synthetischen Aminosäurerest darstellt, wobei die Aminosäurereste, wenn e größer 1 ist, gleich oder verschieden sein können,
$[A^1A^1]$ einen natürlich vorkommenden oder synthetischen Aminosäurerest darstellt, wobei die Aminosäurereste, wenn d größer 1 ist, gleich oder verschieden sein können,
R eine natürlich vorkommende oder synthetische Aminosäure-Seitenkette darstellt,
E eine Hydroxylgruppe oder eine Carboxylschutzgruppe, die in der Peptidsynthese Standard ist, bevorzugt ausgewählt aus Alkoxy, substituiertem Alkoxy oder Aryloxy, oder eine Blockiergruppe, die wie die Carboxylgruppe oder eine Amino- oder substituierte Aminogruppe sein kann, darstellt, wobei die Carboxylschutzgruppe oder Blockiergruppe gegebenenfalls kovalent an einen unlöslichen Polymerträger gebunden sein kann, und die Bogenlinie eine Abstandhaltegruppe der Formel:

$$-(CH_2)_p-(M)_x-Y \qquad (IV)$$

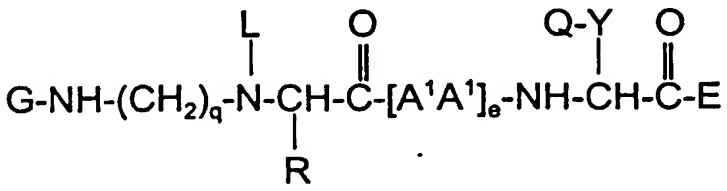
darstellt, worin p wie vorstehend definiert ist und M eine Amino- oder eine Carboxylgruppe oder ein Schwefelatom ist, x gleich 0 oder 1 ist, und Y die Seitenkette einer Hauptkettenaminosäure darstellt, wobei das Verfahren umfaßt:

a) Umsetzen einer Verbindung der Formel:



(XIV)

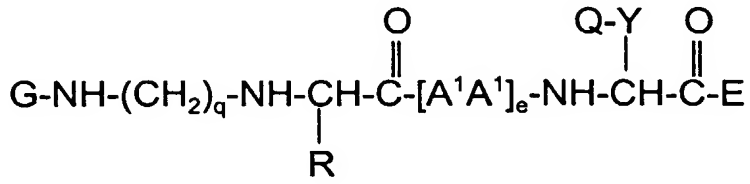
worin E und e wie vorstehend definiert sind, $[A^1A^1]$ einen natürlich vorkommenden oder synthetischen Aminosäurerest darstellt und Y eine Seitenkette einer natürlich vorkommenden oder synthetischen Aminosäure darsteilt und Q eine für die Peptidsynthese gebräuchliche Schutzgruppe darstellt, mit einer Verbindung nach Formel Va, nach Anspruch 1, wobei eine Verbindung der Formel:



(XV)

erhalten wird,

b) selektives Entfernen der Schutzgruppe L von der Verbindung nach Formel XV, wobei eine Verbindung der Formel:



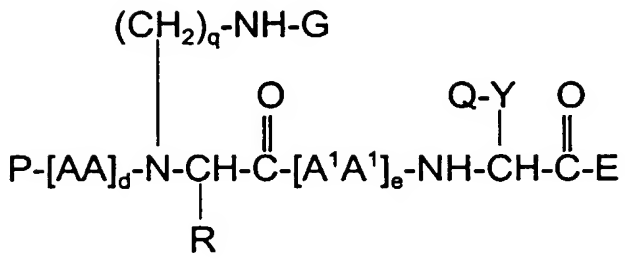
(XVI)

erhalten wird,

c) Umsetzen der Verbindung nach Formel XVI mit einer Aminosäure oder einem Peptid der Formel:

$$P\text{-}[AA]_d\text{-}OH \qquad \text{(XVII)}$$

worin d eine ganze Zahl von 1 bis 10 ist und [AA] einen natürlich vorkommenden oder synthetischen Aminosäurerest darstellt, wobei die Aminosäurereste, wenn d größer 1 ist, gleich oder verschieden sein können, und P eine in der Peptidsynthese gebräuchliche Schutzgruppe darstellt, wobei eine Verbindung der Formel:

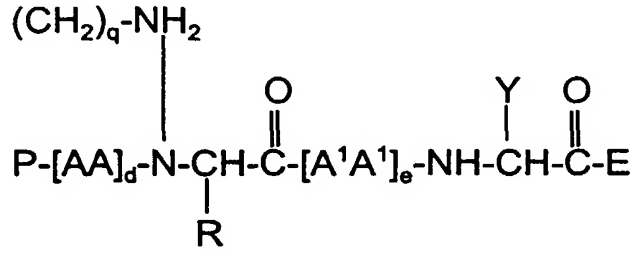


(XVIII)

erhalten wird,

d)

(i) wenn Y eine Seitenkette mit einer Carboxylgruppe ist, selektives Entfernen der Schutzgruppen G und Q von der Verbindung XVIII, wobei eine Verbindung der Formel:



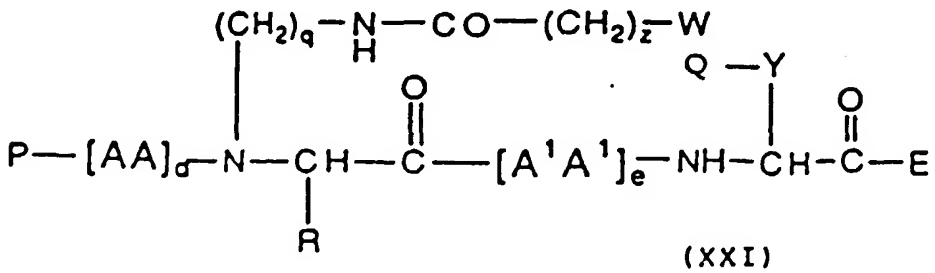
(XIX)

erhalten wird, Cyclisieren der Verbindung XIX, indem sie mit dem Kopplungsmittel gemäß Schritt g) nach Anspruch 1 umgesetzt wird, und Entfernen der Schutzgruppe P und anderer Seitenketten-Schutzgruppen, wobei eine Verbindung der allgemeinen Formet II erhalten wird, oder

(ii) wenn Y eine Seitenkette mit einer von Carboxyl verschiedenen nukleophilen funktionellen Gruppe ist, selektives Entfernen der Schutzgruppe G, Umsetzen der resultierenden Verbindung mit einer Verbindung der Formel:

$$HOOC\text{-}(CH_2)_z\text{-}W \qquad (XX)$$

worin z eine ganze Zahl von 1 bis 10 ist und W eine funktionelle Gruppe ist, die zur Reaktion mit der nukleophilen Gruppe geeignet ist, wobei eine Verbindung der Formel:



(XXI)

erhalten wird, selektives Entfernen der Schutzgruppe Q, woraufhin die Cyclisierung stattfindet, und Entfernen der Schutzgruppe P und anderer Seitenketten-Schutzgruppen von Verbindung XXI, wobei eine Verbindung der allgemeinen Formel II erhalten wird.

7. Verfahren nach Anspruch 6, wobei Y die Seitenkette von Homocystein oder Cystein darsteilt, Q Benzyl ist und E ein Amid ist.

8. Verfahren nach Anspruch 6 oder 7, worin G aus der Gruppe, bestehend aus Z, Boc und Fmoc ausgewählt wird.

9. Verfahren nach Anspruch 6 oder 7, wobei L aus der Gruppe, bestehend aus Z, Boc und Fmoc ausgewählt wird.

10. Verfahren nach Anspruch 9, wobei G Boc ist und L Fmoc ist.

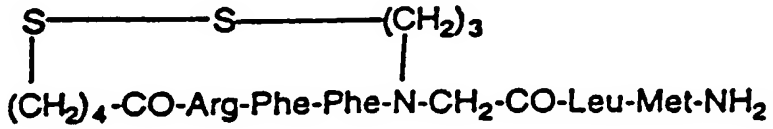
11. Verfahren nach einem der Ansprüche 6 bis 10, wobei in Schritt (c) P Acetyl ist.

12. Verfahren nach einem der Ansprüche 6 bis 10, wobei E ein Amid ist.

13. Verfahren nach einem der Ansprüche 6 bis 11, wobei in Schritt (d) (ii) W aus der Gruppe, bestehend aus Halogenatomen, O-p-Toluolsulfonyl, O-Methansulfonyl und O-Trifluormethansulfonyl ausgewählt wird.

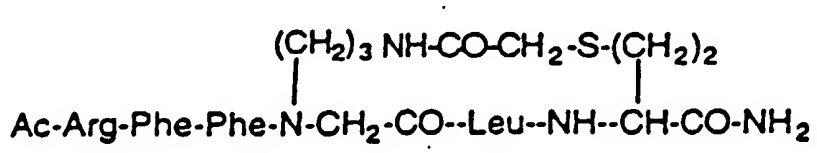
14. Cyclisches, biologisch aktives Polypeptid mit der Formel:

Id

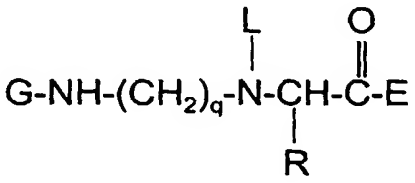


15. Cyclisches, biologisch aktives Polypeptid mit der Formel:

IIa



16. Verfahren zur Herstellung von Verbindungen der allgemeinen Formel Vb:



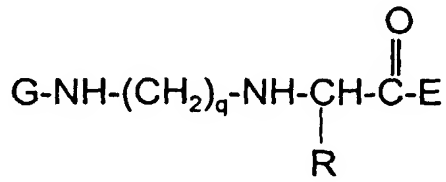
(Vb)

worin q eine ganze Zahl von 2 bis 10 darstellt, G und L, die gleich oder verschieden sein können, jeweils Wasserstoff und/oder eine in der Peptidsynthese gebräuchliche Schutzgruppe darstellen, und E eine Hydroxylgruppe oder eine Carboxylschutzgruppe, die in der Peptidsynthese Standard ist, bevorzugt ausgewählt aus Alkoxy, substituiertem Alkoxy oder Aryloxy, oder eine Blockiergruppe, die wie die Carboxylgruppe oder eine Amino- oder substituierte Aminogruppe sein kann, darstellt, wobei die Carboxylschutzgruppe oder Blockiergruppe gegebenenfalls kovalent an einen unlöslichen Polymerträger gebunden sein kann, durch

(i) Umsetzen der freien Säure Va gemäß Anspruch 1 mit dem entsprechenden, die Gruppe E enthaltenden

Reagenz, wobei der Ester, das Amid oder die kovalente Bindung mit dem polymeren Träger erhalten wird, oder

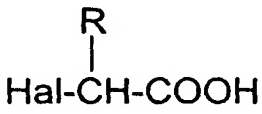
(ii) aus Verbindungen der Formel VIb:



(VIb)

durch Umsetzen dieser freien Säure mit dem entsprechenden, die Gruppe E enthaltenden Reagenz, wobei der Ester, das Amid oder die kovalente Bindung mit dem polymeren Träger erhalten wird, wobei die Verbindung der Formel VIb aus Verbindung XXVI gemäß Anspruch 17 erhalten wird durch Umsetzen dieser freien Säure mit den geeigneten, die Gruppe E enthaltenden Reagenz, wie vorstehend definiert, wobei der Ester, das Amid oder die kovalente Bindung mit dem polymeren Träger erhalten wird.

17. Verfahren zur Herstellung von Verbindungen der allgemeinen Formel Va gemäß Anspruch 1, wobei das Verfahren umfaßt (a) Umsetzen einer α-Halogencarbonsäure der Formel:

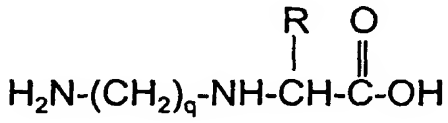


(XXII)

worin R wie in Anspruch 1 definiert ist und Hal Chlor, Brom oder Jod ist, mit einem Alkylendiamin der Formel:

$$H_2N\text{-}(CH_2)_q\text{-}NH_2 \qquad \text{(XXIII)}$$

worin q wie in Anspruch 1 definiert ist, wobei eine Verbindung der Formel:

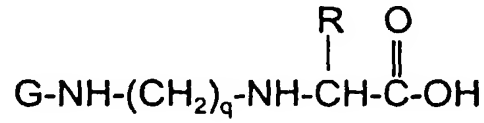


(XXIV)

erhalten wird,

(b)

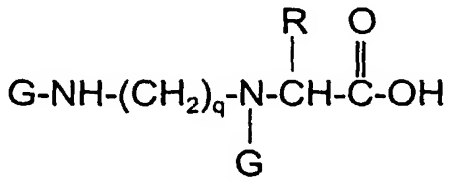
(i) Umsetzen der Verbindung nach Formel XXIV mit dem geeigneten, die Gruppe G enthaltenden Reagenz durch Peptidsynthese-Standardverfahren, wobei G gemäß Anspruch 1 ist, wobei eine Verbindung der Formel



(XXVI)

erhalten wird, und Umsetzen der Verbindung nach Formel (XXVI) mit einem L enthaltenden Reagenz durch Peptidsynthese-Standardverfahren, wobei L in Anspruch 1 definiert ist, wobei eine Verbindung der Formel Va erhalten wird, oder

(ii) Umsetzen der Verbindung nach Formel XXIV mit einem geeigneten, G enthaltenden Reagenz durch Peptidsynthese-Standardverfahren, wobei G wie vorstehend definiert ist, wobei eine Verbindung nach Formel:



(XXVIII)

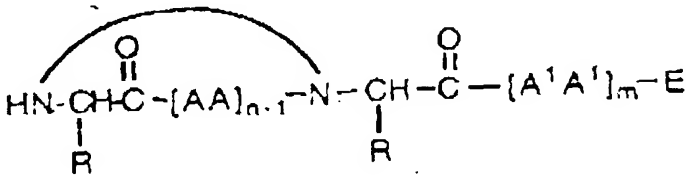
erhalten wird, selektives Entfernen der Schutzgruppe G von der sekundären Aminfunktion, wobei eine Verbindung der allgemeinen Formel XXVI erhalten wird.

18. Pharmazeutische Zusammensetzung, umfassend das Hauptkettencyclisierte Polypeptid gemäß den Ansprüchen 14 oder 15, welche eine beliebige der Verbindungen nach Formel Id oder IIa umfaßt.

19. Pharmazeutische Zusammensetzung nach Anspruch 18 zur Behandlung von Schmerz, Entzündung, Alzheimer-Krankheit, familiärer Dysautonomie, Parkinson-Krankheit und retardiver Dyskinesie.

Revendications

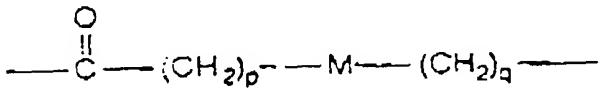
1. Procédé pour la préparation de polypeptides cycliques biologiquement actifs répondant à la formule générale (I)



(I)

dans laquelle

n représente un nombre entier de 1 à 10 ;
m représente un nombre entier de 1 à 10 ; [AA] représente un résidu d'amino-acide naturel ou synthétique, les résidus d'amino-acides, dans le cas où n set supérieur à 1, pouvant être identiques ou différents ;
$[A^1A^1]$ représente un résidu d'amino-acide naturel ou synthétique, les résidus d'amino-acides, dans le cas où m est supérieur à 1, pouvant être identiques ou différents ;
R et R' représentent une chaîne latérale d'amino-acides naturels on synthétiques ;
E représente un groupe hydroxyle, on un groupe protecteur de la fonction carboxyle qui set classique en synthèse de peptides, choisi entre un groupe alkoxy, un groupe alkoxy substitué et un groupe aryloxy, ou un groupe de blocage qui peut être le même que dans le cas du groupe carboxyle on un groupe amino ou amino substitué, le groupe protecteur de la fonction carboxyle ou groupe de blocage pouvant être facultativement fixé par covalence à un support polymérique insoluble, et l'arc de cercle désigne un groupe intercalaire de formula :

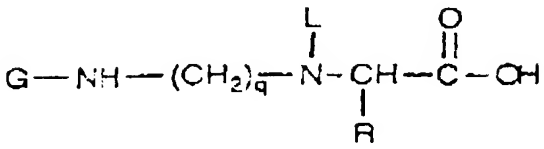


(III)

dans laquelle M est choisi dans le groupe consistant en -S-S-, -CO-NH- et -S- et p et q, qui peuvent être identiques ou différents, représentent chacun un nombre entier de 2 à 10 ;

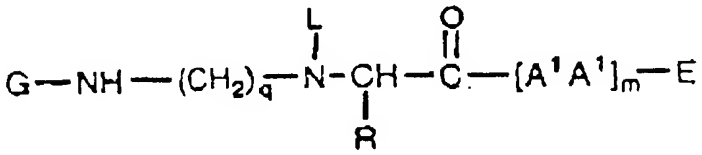
procédé qui comprend les étapes consistant :

(a) à faire réagir un composé de formule :



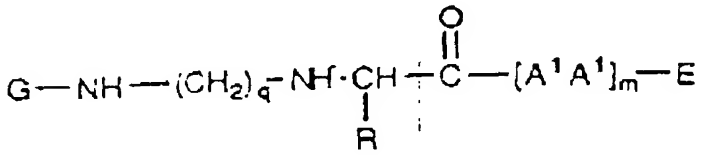
(Va)

dans laquelle q représente un nombre entier de 2 à 10, G et L, qui peuvent être identiques ou différents, représentent chacun un groupe protecteur classique en synthèse de peptides, avec un amino-acide ou un polypeptide de formule H_2N-$[AA]_m$-CO-E, dans laquelle m représente un nombre entier de 1 à 10 et AA rsprésente un résidu d'amino-acide naturel on synthétique, les résidus d'amino-acides, dans le cas où m est supérieur à 1, étant identiques ou différents, et E représente un groupe protecteur de la fonction carboxyle, choisi entre un groupe alkoxy, un groupe alkoxy substitué et un groupe aryloxy, ou un groupe de biocage qui peut être le même que dans le cas du groupe carboxyle ou un groupe amino ou amino substitué, le groupe protecteur de la fonction carboxyle on groupe de blocage pouvant être facultativement fixé par covalence à un support polymérique insoluble, ce qui donne un composé de formule :



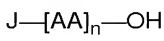
(VIa)

(b) à éliminer de manière sélective du composé de formule VIa le groupe protecteur L, ce qui donne un composé de formule :



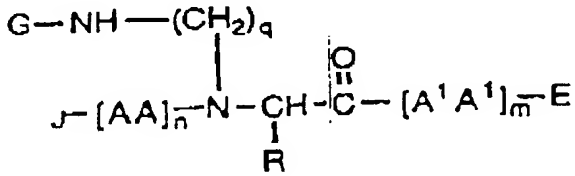
(VIIa)

c) à faire réagir le composé de formule VIIa avec un amino-acide ou peptide de formule :



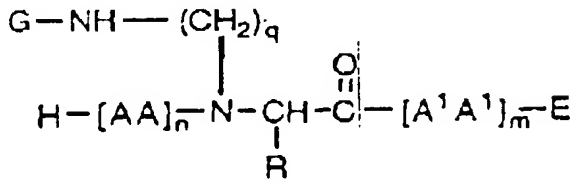
(VIIb)

dans laquelle n représente un nombre entier de 1 à 10, [AA] représente un résidu d'amino-acide naturel ou synthétique, les résidus d'amino-acides, dans le cas où n est supérieur à 1, étant identiques ou différents et J représente un groupe protecteur classique en synthèse de peptides, ce qui donne un composé de formule :



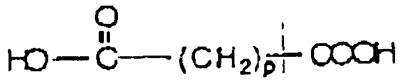
(IXa)

d) à éliminer sélectivement le groupe protecteur J, ce qui donne un composé de formule :



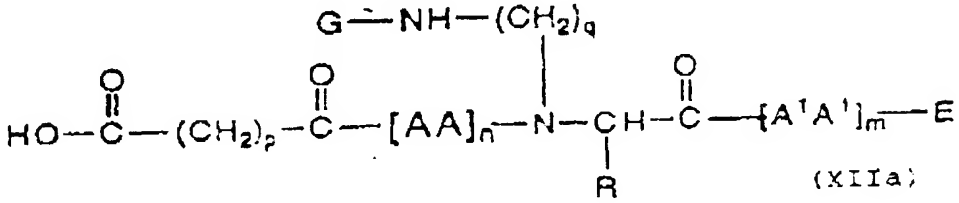
(Xa)

e) à faire réagir le composé de formule Xa avec un composé de formule :



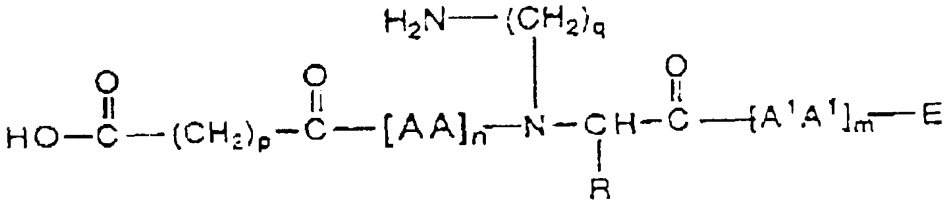
(XIa)

dans laquelle p répond à la définition précitée, ce qui donne un composé de formule :



(XIIa)

f) à éliminer sélectivement du composé XIIa le groupe protecteur G, ce qui donne un composé de formule :



(XIIIa)

g) à faire réagir le composé de formule XIIIa avec un agent de couplage convenable choisi dans le groupe consistant en dicyclohexylcarbodiimide (DCC), chlorure bis(2-oxo-3-oxazolidinyl)phosphinique (BOP-Cl), hexafluorophosphate de benzotriazolyl-N-oxytrisdiméthylaminophosphonium(BOP), 1-oxo-1-chlorophospholane (Cpt-Cl) et un mélange de DCC et d'hydroxybenzotriazole (HOBT), et à éliminer les autres groupes protecteurs de chaîne latérale, ce qui donne un composé de formule générale I dans laquelle m représente un nombre entier de 1 à 10.

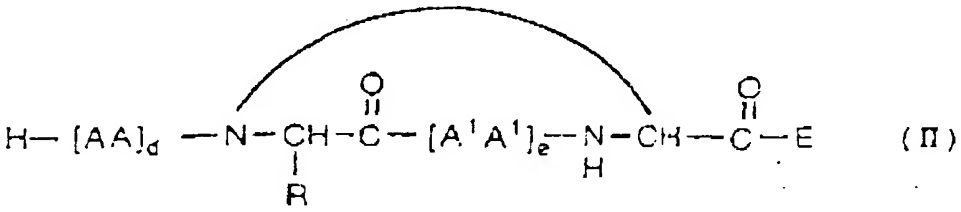
2. Procédé suivant la revendication 1, dans lequel, dans l'étape (a), G set choisi dans le groupe consistant en Z, Boc et Fmoc.

3. Procédé suivant la revendication 1, dans lequel, dans l'étape (a), L est choisi dans le groupe consistant en Z, Boc et Fmoc.

4. Procédé suivant les revendications 2 et 3, dans lequel G représente Boc et L représente Fmoc.

5. Procédé suivant les revendications 2 et 3, dans lequel G représente Fmoc et L représente Boc.

6. Procédé pour la préparation de polypeptides cyciisés biologiquement actifs de formule générale (II)



(II)

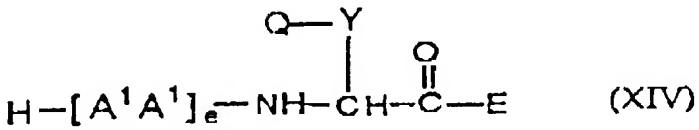
dans laquelle

d est égal à 0 ou à un nombre entier de 1 à 10 et e représente un nombre entier de 1 à 10 ;
[AA] représente un résidu d'amino-acide naturel ou synthétique, les résidus d'amino-acides, dans le cas où e est supérieur à 1, pouvant être identiques ou différents ;
$[A^1A^1]$ représente un résidu d'amino-acide naturel ou synthétique, les résidus d'amino-acides, dans le cas où d est supérieur à 1, pouvant être identiques ou différents ;
R représente une chaîne latérale d'amino-acides naturels ou synthétiques ;
E représente un groupe hydroxyle, ou un groupe protecteur de la fonction carboxyle qui est classique en synthèse de peptides, choisi de préférence entre in groupe alkoxy, un groupe alkoxy substitué et un groupe aryloxy, ou un groupe de blocage qui peut être le même que dans le cas du groupe carboxyle ou un groupe amino ou amino sutstitué, le groupe protecteur de la fonction carboxyle ou groupe de biocage pouvant être facultativement fixé par covalence à un support polymérique insoluble, et l'arc de cercle désignant un groupe intercalaire de formule :

$$—(CH_2)_p—(M)_x—Y \qquad (IV)$$

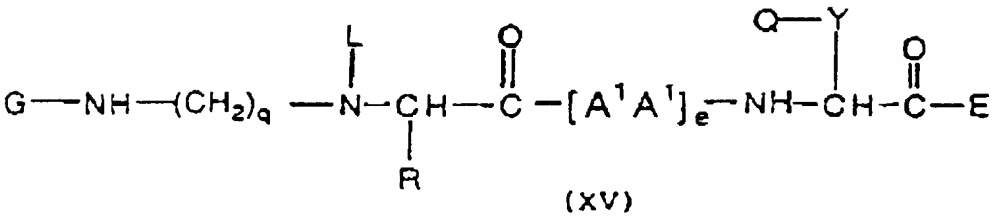
dans laquelle p répond à la définition précitée et M représente un groupe amino ou carboxyle ou un atome de soufre, x est égal à 0 ou 1 et Y représente la chaîne latérale d'un amino-acide de squelette, procédé qui comprend les étapes consistant :

a) à faire réagir un composé de formule :



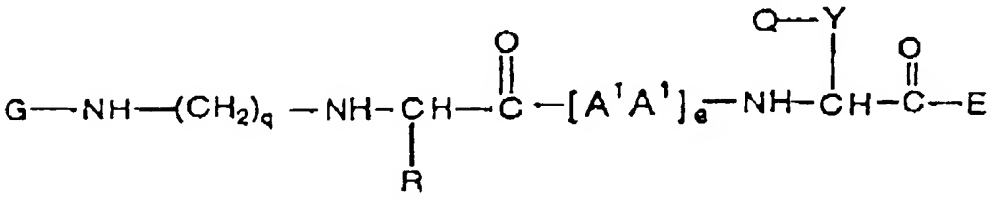
(XIV)

dans laquelle E et e répondent aux définitions précitées, $[A^1A^1]$ représente un résidu d'amino-acide naturel ou synthétique et Y représente une chaîne latérale d'un amino-acide naturel ou synthétique et Q représente un groupe protecteur classique en synthèse de peptides, avec un composé de formule Va suivant la revendication 1, ce qui donne un composé de formule :



(XV)

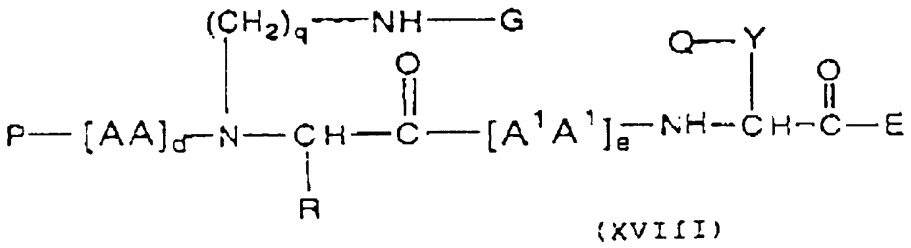
b) à éliminer sélectivement du composé de formule XV le groupe protecteur L, ce qui donne un composé de formule :



c) à faire réagir le composé de formule XVI avec un amino-acide ou peptide de formule :

$$P—[AA]_d—CH \qquad (XVII)$$

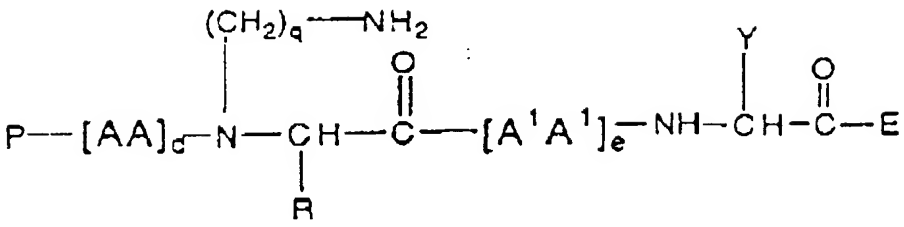
dans laquelle d représente un nombre entier de 1 à 10 et [AA] représente an résidu d'amino-acide naturel ou synthétique, les résidus d'amino-acides, dans le cas où d est supérieur à 1, étant identiques ou différents, et P représente un groupe protecteur classique en synthèse de peptides, ce qui donne un composé de formule :



(XVIII)

d)

(i) dans le cas où Y représente une chaîne latérale portant un groupe carboxyle, à éliminer sélectivement les groupes protecteurs G et Q du composé XVIII, ce qui donne un composé de formule :



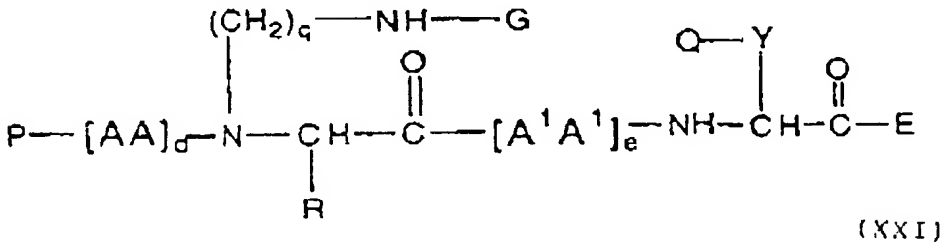
(XIX)

à cycliser le composé XIX en le faisant réagir avec l'agent de couplage suivant l'étape g) de la revendication 1 et à éliminer le groupe protecteur P et les autres groupes protecteurs de chaîne latérale, ce qui donne un composé de formule générale II ; ou

(ii) dans le cas où Y représente une chaîne latérale portant un groupe fonctionnel nucléophile autre qu'un groupe carboxyle, à éliminer sélectivement le groupe protecteur G, à faire réagir le composé résultant avec un composé de formule :

$$HOOC—(CH_2)_z—W \qquad (XX)$$

dams laquelle z représence un nombre entier de 1 à 10 et W représente un groupe porteur de fonction capable de réagir avec ledit groupe nucléophile, ce qui donne un composé de formule :



(XXI)

à éliminer sélectivement le groupe protecteur Q, la cyclisation se produisant, et à éliminer le groupe protecteur P et les autres groupes protecteurs de chaîne latérale du composé XXI, ce qui donne un composé de formule générale II.

7. Procédé suivant la revendication 6, dans lequel Y représente la chaîne latérale de l'homocystéine ou de la cystéine, Q représente un groupe benzyle et E représente un amide.

8. Procédé suivant la revendication 6 ou la revendication 7, dans lequel G est choisi dans le groupe consistant en Z, Boc et Fmoc.

9. Procédé suivant la revendication 6 ou la revendication 7, dans lequel L est choisi dans le groupe consistant en Z, Boc et Fmoc.

10. Procédé suivant la revendication 9, dans lequel G représente Boc et L représente Fmoc.

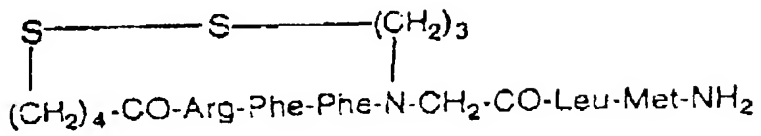
11. Procédé suivant l'une quelconque des revendications 6 à 10, dans lequel, dans l'étape (c), P représente un groupe acétyle.

12. Procédé suivant l'une quelconque des revendications 6 à 10, dans lequel E représente un amide.

13. Procédé suivant l'une quelconque des revendications 6 à 11, dans lequel, dans l'étape (d) (ii), W est choisi dans le groupe consistant en des atomes d'halogènes, un groupe O-p-toluènesulfonyle, un groupe O-méthanesulfonyle et un groupe O-trifluorométhanesulfonyle.

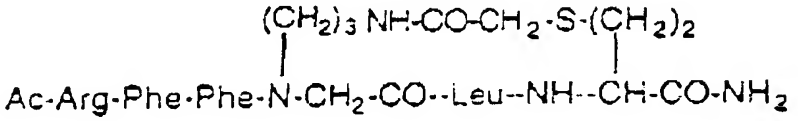
14. Polypeptide cyclique biologiquement actif répondant à la formule :

Id

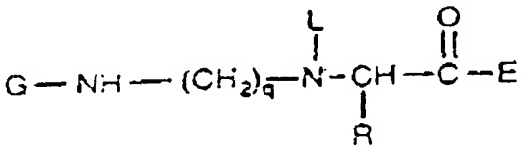


15. Polypeptide cyclique biologiquement actif répondant à la formule :

IIa



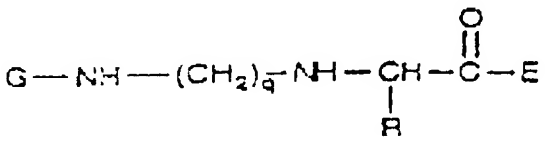
16. Procédé pour la préparation de composés répondant à la formule générale Vb :



(Vb)

dans laquelle q représente un nombre entier de 2 à 10, G et L, qui peuvent être identiques ou différents, représentent chacun l'hydrogène et/ou un groupe protecteur classique en synthèse de peptides, et E représente un groupe hydroxyle, ou un groupe protecteur dans la fonction carboxyle qui est classique en synthèse de peptides, choisi die préférence entre un groupe alkoxy, un groupe alkoxy substitué et un groupe aryloxy, ou un groupe de blocage qui peut être le même que dans le cas du groupe carboxyle on un groupe amino on amino substitué, la groupe protecteur de la fonction carboxyle on groupe de blocage pouvant être facultativement fixé par covalence à un support polymérique insoluble,

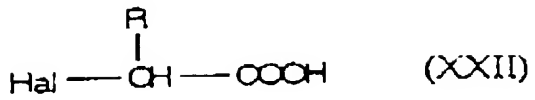
(i) en faisant réagir l'acide libre Va suivant la revendication 1 avec le réactif approprié contenant le groupe E, ce qui donne la liaison ester, la liaison amide ou la liaison covalente avec le support polymérique ; ou
(ii) à partir de composés de formule VIb :



(VIb)

en faisant réagir cet acide libre avec le réactif approprié contenant le groupe E, ce qui donne la liaison ester, la liaison amide ou la liaison covalente avec le support polymérique ; le composé de formula VIb étant obtenu à partir du composé XXVI suivant la revendication 17 en faisant réagir cet acide libre avec le réactif approprié contenant le groupe E répondant à la définition précitée, ce qui donne la liaison ester, la liaison amide ou la liaison covalente avec le support polymérique.

17. Procédé pour la préparation de composés de formula générale Va, suivant la revendication 1, procédé qui comprend (a) la réaction d'un acide α-halogénocarboxylique de formule :

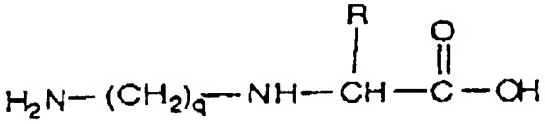


(XXII)

dans laquelle R répond à la définition suivant la revendication 1 et Hal représente le chlore, le brome ou l'iode, avec une alkylène-diamine de formule :

(XXIII)

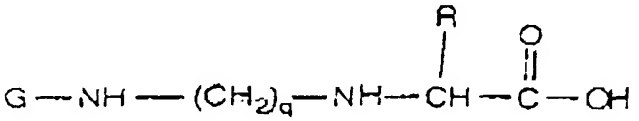
dans laquelle q répond à la définition suivant la revendication 1, ce qui donne un composé de formule :



(XXIV)

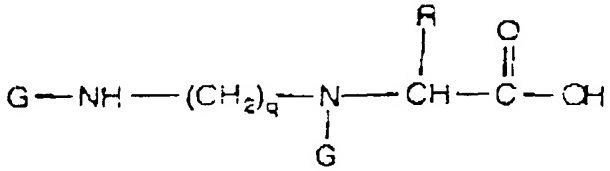
(b)

(i) la réaction du composé de formule XXIV avec un réactif convenable contenant le groupe G par des procédés classiques en synthèse de peptides, G répondant à la définition suivant la revendication 1, ce qui donne un composé de formule :



(XXVI)

et la réaction du composé de formule XXVI avec un réactif contenant L par des procédés classiques en synthèse de peptides, L répondant à la définition suivant la revendication 1, ce qui donne un composé de formule Va, ou
(ii) la réaction du composé de formule XXIV avec un réactif convenable contenant G par des procédés classiques en synthèse de peptides, G répondant à la définition précitée, ce qui donne un composé de formule :



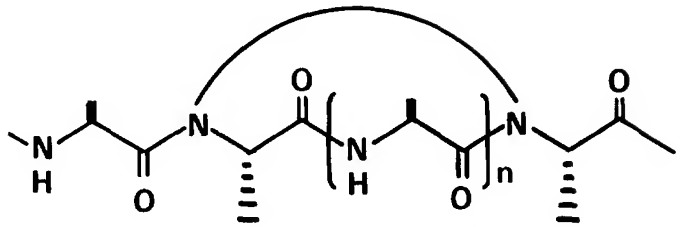
(XXVIII)

et l'elimination sélective du groupe protecteur G de la fonction amino secondaire, ce qui donne un composé de formule générale XXVI.

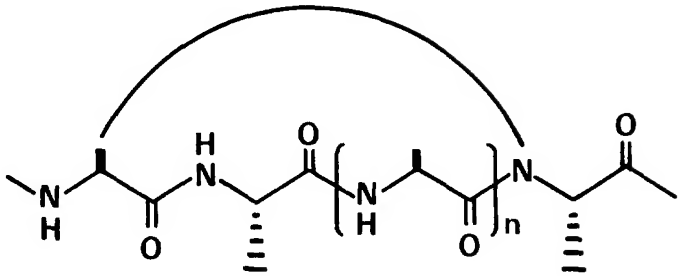
18. Composition pharmaceutique, comprenant le polypeptide cyclisé an niveau du squelette suivant la revendication 14 ou 15, qui comprend l'un quelconque des composés de formule Id ou IIa.

19. Composition pharmaceutique suivant la revendication 18 pour le traitement de la douleur, de l'inflammation, de la maladie d'Alzheimer, de la dysautonomie familiale, de la maladie de Parkinson et de la dyskinésie tardive.

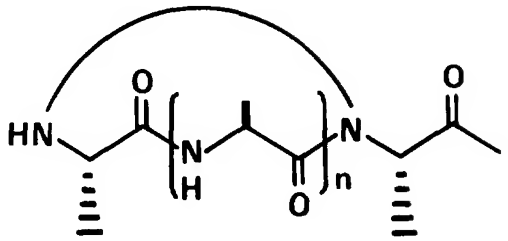
FIG. 1. THE CONCEPT OF N-BACKBONE CYCLIZATION.

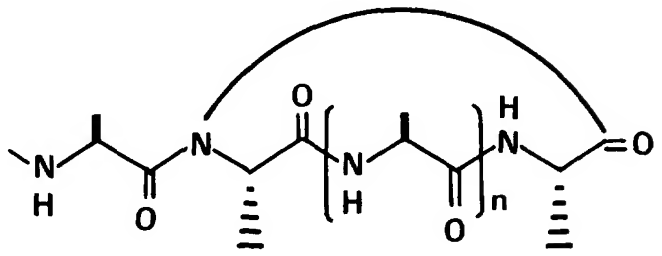


a N to N-backbone



b N-backbone to side chain.

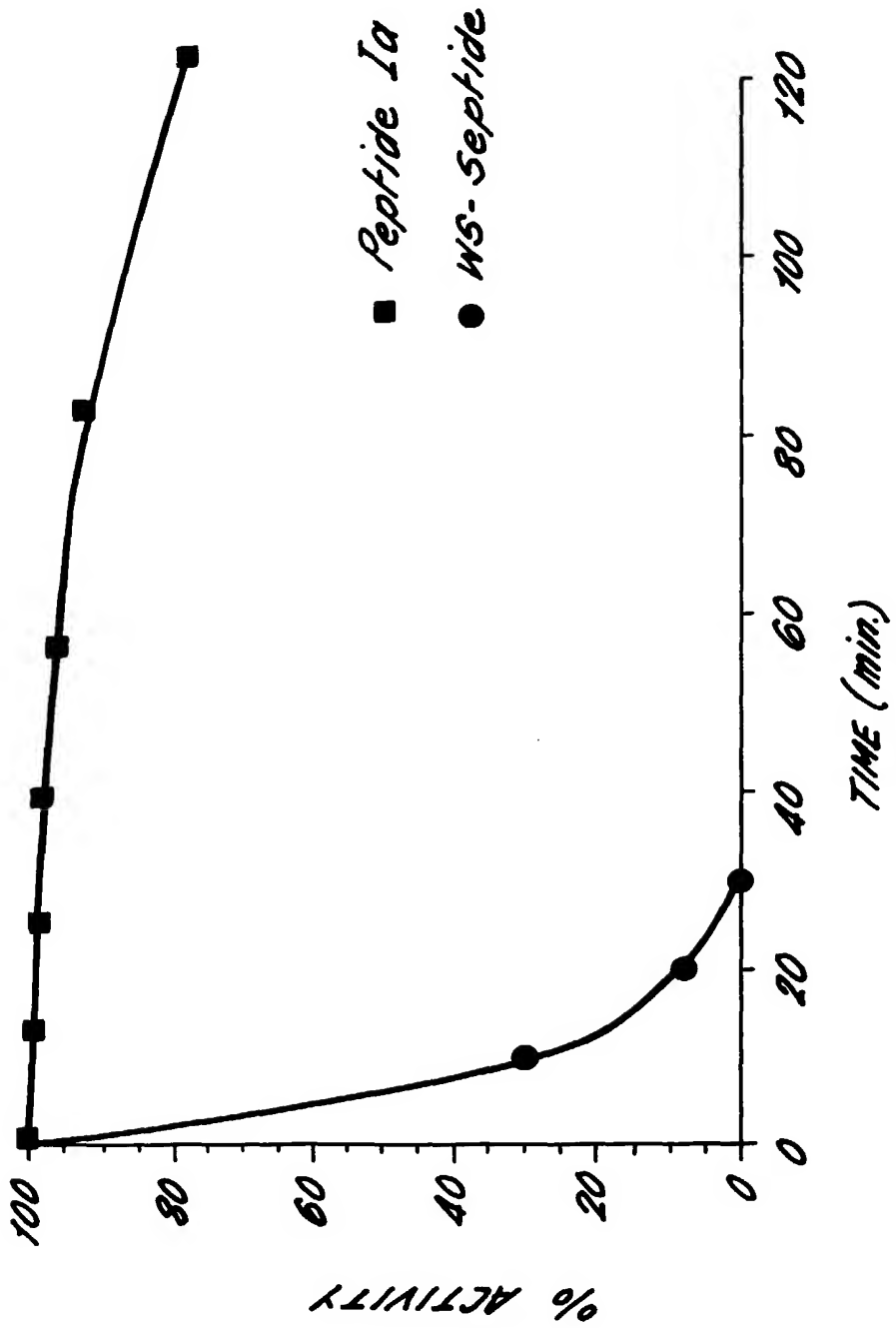




c N-backbone to end

⌒ = -R-S-S-R-, -R-CO-NH-R-, -R-CH_2-S-R-, etc.

FIG. 2.



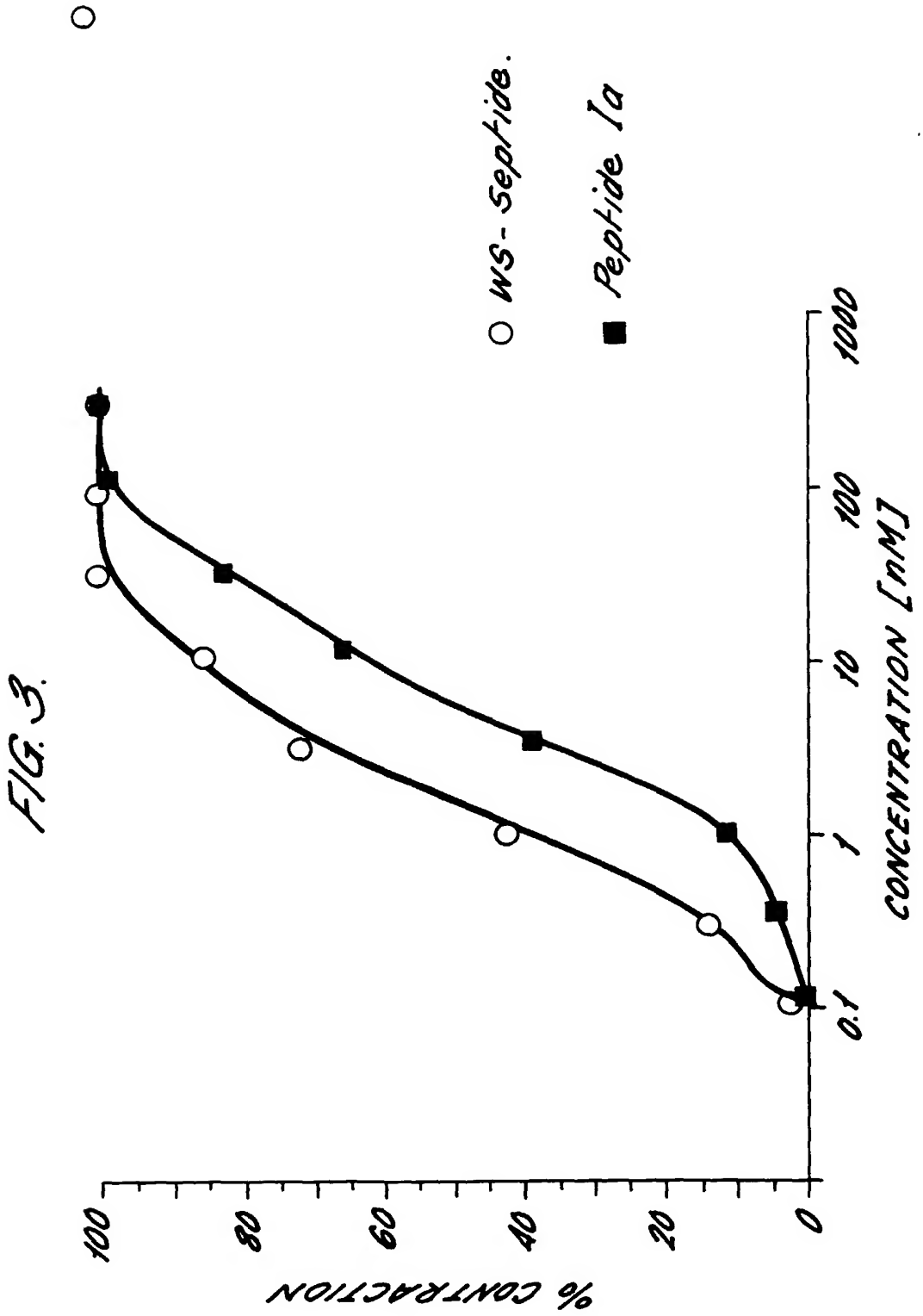

WS-Sepfide.
Peptide Ia
% CONTRACTION
0
20
40
60
80
100
0.1
1
10
100
1000
CONCENTRATION [nM]

FIG. 3.